The Clorox Company
Technical Center

January 4, 1992

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street S.W.
Washington, D.C. 20460

Attention: Section 8(e) Coordinator (CAP Agreement)
Re: CAP Agreement 0007

Clorox is a distributor, in commerce, of a product containing 2-butoxyethanol, CAS # 111-76-2. Clorox is also a participant in EPA's Compliance Audit Program.

On June 24, 1992, Clorox filed an 8(e) notice on 2-butoxyethanol based on preliminary results from short term toxicity studies conducted by the National Toxicology Program. The preliminary data from these studies described a number of new reproductive effects that were attributed to 2-butoxyethanol including reduced epididymal sperm density in male rats and reduced uterine weight and altered frequency of estrous cycle stages in female rats.

We recently requested a copy of the draft technical report from these studies. Based on our review of the report, there is no new reportable substantial risk information. As a follow-up to our original 8(e) notification, however, I have enclosed a copy of the draft for your review.

If you have any questions, please feel free to call me at (510) 847-6249.

Very truly yours,

William C. McCormick
Research Associate

attachments

mm
2/9/95

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National Toxicology Program
Toxicity Report Series
Number 26

Draft NTP Technical Report on Toxicity Studies of

Ethylene Glycol Ethers

2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol

(CAS Nos. 109-86-4, 110-80-5, 111-76-2)

Administered in Drinking Water to F344/N Rats and B6C3F$_1$ Mice

Scheduled Peer Review Date: December 1-2, 1992

Michael P. Dieter, PhD, Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NOTICE

This **DRAFT** toxicity report has been prepared for public review and comment. Until a future **DRAFT** of this report has been reviewed and approved by the National Toxicology Program's Board of Scientific Counselors' Peer Review Panel in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact the National Toxicology Program's Public Information Office for the final version of this report.

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. NTP coordinates the relevant Public Health Service programs, staff, and resources that are concerned with basic and applied research and with biological assay development and validation.

NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

To carry out its mission, NTP designs and conducts studies to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential.

The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

Single copies of this report are available without charge, while supplies last, from the NTP Public Information Office (telephone number 919/541-3991).

NTP Public Information Office
NIEHS
Post Office Box 12233
Research Triangle Park, NC 27709

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United States Department of Health and Human Services
Public Health Service
National Institutes of Health

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This NTP report on the toxicity studies of ethylene glycol ethers is based primarily on 2-week, 13-week, and stop-exposure studies conducted in 1988 at EG&G Mason Research Institute, Worcester, MA.

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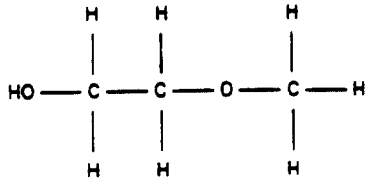
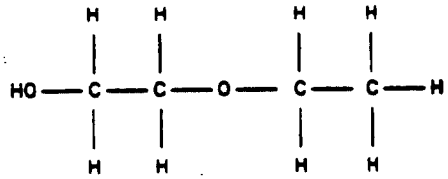
2-Methoxyethanol



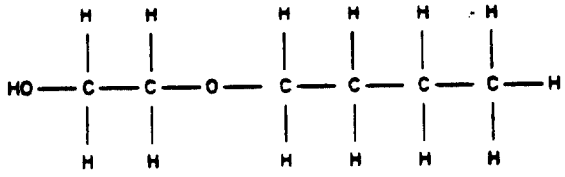
Molecular Formula $C_3H_8O_2$
CAS Number 109-86-4
Molecular Weight 76.10
Synonyms Ethylene Glycol Monomethyl Ether, Methyl Cellosolve

2-Ethoxyethanol



Molecular Formula $C_4H_{10}O_2$
CAS Number 110-80-5
Molecular Weight 90.12
Synonyms Ethylene Glycol Monoethyl Ether, Cellosolve

2-Butoxyethanol



Molecular Formula $C_6H_{14}O_2$
CAS Number 111-76-2
Molecular Weight 118.17
Synonyms Ethylene Glycol Monobutyl Ether, Butyl Cellosolve

Glycol alkyl ethers represent a class of high-production-volume chemicals with widespread industrial application as solvents and chemical intermediates. Comparative toxicity studies with 3 glycol ethers, 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol, were conducted in F344/N rats and B6C3F$_1$ mice in both 2-week and 13-week drinking water studies. Toxicologic endpoints for evaluation in animals included histopathology, clinical chemistry, hematology, and urinalysis, and reproductive system toxicity. Genetic toxicity was also evaluated for each glycol ether in several *in vitro* and *in vivo* assays.

In the 2-week studies, groups of 5 male and 5 female rats and mice received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water at concentrations ranging from 200 to 1200 mg/kg, 300 to 2500 mg/kg, and 100 to 650 mg/kg, respectively. There were no chemical-related effects on survival for rats or mice in these studies. Decreased body weight gains were noted for both male and female rats treated with 2-methoxyethanol or 2-ethoxyethanol for 2 weeks, and there were dose-related decreases in water consumption for rats of each sex treated with the ethylene glycol ethers. Most of the changes in organ weights in rats and mice treated with the glycol ethers were sporadic (mice) or related to low final mean body weights (rats), except for thymic atrophy in males and females and testicular atrophy in males of both species. In many cases, these responses were dose related, and the relative degree of toxicity to the thymus and testis was ranked as 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol.

In the 13-week studies in rats, groups of 10 males and 10 females received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water at concentrations ranging from 750 to 6000 ppm, 1250 to 20,000 ppm, and 750 to 6000 ppm, respectively. In the 13-week studies in mice, groups of 10 males and 10 females received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water at concentrations ranging from 2000 to 10,000 ppm, 2500 to 40,000 ppm, and 750 to 6000 ppm, respectively. Chemical-related mortality occurred in male and female rats administered 4500 or 6000 ppm 2-methoxyethanol and in male and female rats administered 20,000 ppm 2-ethoxyethanol. No deaths occurred in rats administered 2-butoxyethanol or in mice administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol. Decreased body weight gains occurred in dosed rats and mice in all 3 studies; the greatest reductions in body weight gain were seen with 2-methoxyethanol.

In rats administered 2-methoxyethanol or 2-ethoxyethanol, treatment-related histopathologic changes were observed in the testes, thymus, and hematopoietic tissues (spleen, bone marrow, and liver). A dose-related degeneration of the germinal epithelium in the seminiferous tubules of the testes was more severe in 2-methoxyethanol-treated rats than in rats treated with 2-ethoxyethanol. In special stop-exposure studies in male rats in which administration of the glycol ethers was stopped after 60 days, only partial recovery from testicular degeneration was noted after a 56-day recovery period in rats treated with 2-methoxyethanol or 2-ethoxyethanol.

2-Methoxyethanol treatment for 13 weeks resulted in a progressive anemia associated with a cellular depletion of bone marrow and fibrosis of the splenic capsule. Anemia was also seen with 2-ethoxyethanol, but evidence of an adaptive response was indicated by increased hematopoiesis in the bone marrow, spleen, and liver. Toxicity with 2-butoxyethanol was limited to the liver and hematopoietic system. Cytoplasmic alteration and a minimal hepatocellular degeneration were present in the liver of male and female rats. A minimal anemia was present, and a hematopoietic response was evident in the bone marrow and spleen.

In mice, 2-methoxyethanol and 2-ethoxyethanol had similar effects on the testes, spleen, and adrenal gland (females only). A dose-related degeneration of the germinal epithelium in seminiferous tubules of the testes was more severe with 2-methoxyethanol than with 2-ethoxyethanol. A dose-related increase in splenic hematopoiesis was also more prominent with 2-methoxyethanol. Both 2-methoxyethanol and 2-ethoxyethanol caused a prominent lipid vacuolization of the X-zone of the adrenal gland in female mice. There were no chemical-related lesions attributed to 2-butoxyethanol administration in mice.

All 3 of the glycol ethers were negative in *Salmonella typhimurium* mutation tests conducted with and without induced hamster and rat liver S9. In the mouse lymphoma L5178Y cell mutation assay, 2-ethoxyethanol was negative without S9 but was weakly positive in the presence of induced rat liver S9; 2-methoxyethanol and 2-butoxyethanol were not tested in this assay. At high concentrations, 2-ethoxyethanol induced sister chromatid exchanges (SCEs) in Chinese hamster ovary cells, with and without S9. Chromosomal aberrations (Abs) were also induced by 2-ethoxyethanol, but only in the

absence of S9 and without a delay in cell cycle. In contrast, 2-butoxyethanol induced cell cycle delay but did not induce either SCEs or Abs, with or without S9. 2-Ethoxyethanol was the only glycol ether tested for induction of sex-linked recessive lethal mutations in germ cells of *Drosophila melanogaster*, and both feeding and injection trials were negative.

In summary, the rank order of toxicity for the 3 glycol alkyl ethers was 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol. Based on survival, decreased body weight gains, and histopathological effects for each glycol ether tested, the toxicity was greater in rats than in mice. The major target organs for toxicity were the testes in male rats and mice and the hematopoietic system in rats and mice of each sex.

In the 13-week study of 2-methoxyethanol in rats, a no-observed-adverse-effect level (NOAEL) was not reached, since testicular degeneration in males and decreased thymus weights in males and females occurred at the lowest concentration administered (750 ppm). In the 13-week study of 2-ethoxyethanol in rats, the NOAEL for testicular degeneration and decreased thymus weights in males was 1250 ppm; for female rats treated with 2-ethoxyethanol for 13 weeks, the NOAEL for all histopathologic and hematologic effects was 5000 ppm. An NOAEL for rats treated with 2-butoxyethanol for 13 weeks was not reached, since cytoplasmic alteration in the liver occurred in males and females at the lowest concentration administered (750 ppm).

For male mice treated with 2-methoxyethanol for 13 weeks, the NOAEL for testicular degeneration and increased hematopoiesis in the spleen was 2000 ppm. An NOAEL was not reached for female mice treated with 2-methoxyethanol, since adrenal gland hypertrophy and increased hematopoiesis in the spleen occurred at the lowest concentration administered (2000 ppm). For male mice treated with 2-ethoxyethanol for 13 weeks, the NOAEL for testicular degeneration and increased hematopoiesis in the spleen was 20,000 ppm. For female mice in the 13-week study of 2-ethoxyethanol, the NOAEL for adrenal gland hypertrophy and increased hematopoiesis in the spleen was 5000 ppm. No clear chemical-related effects were seen in male or female mice administered 2-butoxyethanol for 13 weeks at concentrations as high as 6000 ppm.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of ethylene glycol ethers on December 1-2, 1992 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies are appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

NOTE: This section will be completed in a future draft of this toxicity report.

INTRODUCTION

Chemical and Physical Properties, Production, Use, and Exposure

Three of the simplest glycol alkyl ethers, 2-methoxyethanol (methyl Cellosolve® or ethylene glycol monomethyl ether), 2-ethoxyethanol (Cellosolve® or ethylene glycol monoethyl ether), and 2-butoxyethanol (butyl Cellosolve® or ethylene glycol monobutyl ether) are colorless organic liquids with a mild, non-residual odor, a sweetish odor, or a mild ether odor and with odor thresholds of 2.3, 2.7, and 0.10 ppm, respectively (Amoore and Hautala, 1983). They are miscible with water and many organic solvents. Chemical and physical properties for the 3 compounds are listed in Table 1.

TABLE 1 Chemical and Physical Properties of the Ethylene Glycol Ethers[1]

Parameter	2-Methoxyethanol	2-Ethoxyethanol	2-Butoxyethanol
Specific gravity	0.962	0.926	0.898
Boiling point (°C)	124.2	135.0	170.8
Freezing point (°C)	–85	–100	–77
Vapor pressure (mm Hg at 25°C)	9.7	5.75	0.88
Refractive index	1.400	1.406	1.417
Flash point (°C), closed cup	39	43	62
Autoignition temperature (°C)	285	235	238
Flammability limits (vol. % in air)	1.8–14.0	1.70–15.6	1.10–12.7
Water solubility	miscible	miscible	miscible
Vapor density (air=1)	2.6	3.1	4.1
ppm in saturated air (25°C)	12,800	7,600	1,200

[1] Adapted from NIOSH (1990, 1991).

The 3 glycol alkyl ethers are produced by reaction of ethylene oxide with their respective alcohols or by direct alkylation of ethylene oxide with agents like dimethyl, diethyl, or dibutyl sulfate (Rowe and Wolf, 1982). The products of these reactions are not pure glycol alkyl ethers. The glycol alkyl ethers must be separated from diethers and higher glycols.

2-Methoxyethanol is used as a jet fuel deicer (Meridian Research, Inc., 1987), as a plasticizer, and in the manufacture of printed circuit boards; it is also used in ink, photography, and dyeing applications. 2-Ethoxyethanol is used as a solvent and a chemical intermediate for the synthesis of ethylene glycol monoethyl ether acetate. 2-Butoxyethanol is used as a solvent, chemical intermediate, and component of herbicides and brake fluid. A complete review of the uses of these glycol alkyl ethers can be found in 2 National Institute for Occupational Safety and Health (NIOSH) criteria documents, 1 for 2-butoxyethanol (1990) and the other for 2-methoxyethanol and 2-ethoxyethanol (1991).

Because of the widespread applications of the glycol alkyl ethers and their large annual production volume, large numbers of U.S. workers are potentially exposed. For example, over the last 5 years, about 70 million pounds of 2-methoxyethanol, 110 million pounds of 2-ethoxyethanol, and 350 million pounds of 2-butoxyethanol were produced (SRI, 1992). For detailed exposure data, refer to tables 3-3 to 3-5 in NIOSH criteria documents 90-118 (1990) and 91-119 (1991).

Absorption, Disposition, Metabolism, and Excretion

The metabolism of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol has been investigated in rats, rabbits, guinea pigs, and dogs. In several studies, the 3 glycol alkyl ethers were shown to undergo oxidization catalyzed by alcohol dehydrogenase to intermediate aldehydes, which then underwent further oxidation catalyzed by aldehyde dehydrogenase to their respective acids (Carpenter *et al.*, 1956; Jonsson and Steen, 1978; Jonsson *et al.*, 1982; Miller *et al.*, 1983a,b; Cheever *et al.*, 1984; Moss *et al.*, 1985). The acid metabolites were found in the urine; in the case of 2-ethoxyethanol, some of the ethoxyacetic acid was conjugated with glycine to form *N*-ethoxyacetyl glycine (Jonsson *et al.*, 1982; Cheever *et al.*, 1984). Subsequent investigations using radiolabeled 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol administered to rats in the drinking water revealed another metabolic product, ethylene glycol, in the urine. These studies also demonstrated that the fraction of the dose metabolized to ethylene glycol and carbon dioxide was inversely proportional to chain length (Medinsky *et al.*, 1990). This result was confirmed in an inhalation study of 2-butoxyethanol in rats

(Sabourin *et al.*, 1992a). The elimination of 10% to 20% of the dose of each glycol alkyl ether as ethylene glycol in the urine suggested that dealkylation prior to oxidation to the alkoxyacetic acids could occur, which would represent an alternate pathway of metabolism and avoid formation of acid metabolites.

Additionally, metabolism studies of all 3 glycol alkyl ethers were conducted after human inhalation exposures, and the presence of the respective alkoxyacetic acids in the urine was confirmed (Groeseneken *et al.*, 1986a,b, 1987, 1988, 1989; Johanson *et al.*, 1986, 1988). These investigations also demonstrated that the half-life of 2-methoxyacetic acid in humans was greater than 70 hours (compared to about 12 hours in pregnant rats and 20 hours in pregnant macaque monkeys), and that dermal exposure to 2-butoxyethanol resulted in systemic uptake and the appearance of butoxyacetic acid in the urine.

Toxicity

ANIMAL TOXICITY

Glycol alkyl ethers exhibit a spectrum of toxicity, dependent upon dose, carbon chain length, route of exposure, and species investigated. Reviews of the toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were published by NIOSH in 1990 and 1991. Alkyoxacetic acids are the primary metabolites of the ethylene glycol mono-*n*-alkyl ethers and are considered to be the toxic agents (Ghanayem *et al.*, 1989). The target organs and systems that exhibited toxicity to these compounds included the kidney, liver, hematopoietic system, central nervous system, and reproductive system. The primary focus for the toxicity of glycol alkyl ethers is any population of rapidly dividing cells, such as embryonic stem cells (Nagano *et al.*, 1981), bone marrow stem cells (Hong *et al.*, 1988, 1989), tumor cells (Houchens *et al.*, 1984; Dieter *et al.*, 1990), renal tubule cells (Karel *et al.*, 1947; Dodd *et al.*, 1983), and spermatocytes (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987). However, there is a remarkable specificity in the toxicity of these 3 glycol alkyl ethers. For example, 2-methoxyethanol is a potent teratogen (Nagano *et al.*, 1981; Horton *et al.*, 1985; Greene *et al.*, 1987; Feuston *et al.*, 1990), but it exhibits little of the erythrocytic hemolysis produced by 2-butoxyethanol (Bartnik *et al.*, 1987; Ghanayem *et al.*, 1987a; Ghanayem, 1989); these toxic effects are seen with 2-ethoxyethanol only at higher doses. 2-Methoxyethanol and 2-ethoxyethanol

are potent spermatotoxins (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987) while 2-butoxyethanol is ineffective in this capacity. 2-Methoxyethanol was more than twice as effective as 2-ethoxyethanol in delaying tumor progression in a leukemia transplant model, and it was equally more potent in reducing the number of mouse, rat, or human leukemia cells in culture. 2-Butoxyethanol, on the other hand, was ineffective whether tested *in vivo* or *in vitro* in this system (Dieter *et al.*, 1990).

HUMAN TOXICITY

As early as 1936 and 1938, case studies were reported in which exposure to 2-methoxyethanol occurred in shirt factories (Donley, 1936; Parsons and Parsons, 1938). Occupational exposure of humans to 2-methoxyethanol dermally or by inhalation in printing (Groetschel and Schuermann, 1959; Zavon, 1963), electroplating (Ohi and Wegman, 1978), and microfilm production operations (Cohen, 1984) induced reversible toxicity that affected the hematopoietic and central nervous systems. An accidental poisoning resulted in reversible renal toxicity in 2 men who ingested pure 2-methoxyethanol (Nitter-Hauge, 1970). One case was reported in which a woman ingested 40 mL 2-ethoxyethanol, resulting in toxicity to the central nervous system, liver, and kidneys that persisted for up to 1 year (Fucik, 1969). In 2 other cases, women attempted suicide by ingesting 2-butoxyethanol, which resulted in reversible hematotoxicity (Rambourg-Schepens *et al.*, 1988; Gijsenbergh, 1989). In a study conducted in the 1950s, 4 men and 3 women were voluntarily exposed by inhalation to 2-butoxyethanol for as long as 8 hours at a concentration of 100 ppm or for 2 4-hour exposure periods at concentrations as great as 200 ppm. In this study, inhalation exposure to 2-butoxyethanol resulted in various symptoms of toxicity, including nose and throat irritation, headaches, and vomiting; erythrocyte osmotic fragility was unchanged (Carpenter *et al.*, 1956). Butoxyacetic acid was excreted in the urine of the subjects.

Numerous epidemiological studies of glycol alkyl ethers were also conducted from 1938 to 1988. These studies investigated exposure to 2-methoxyethanol in shirt factories (Greenburg *et al.*, 1938) as well as in manufacturing and packaging operations (Cook *et al.*, 1982), exposure to 2-ethoxyethanol in the preparation of ceramic molds (Ratcliffe *et al.*, 1986), and exposure to 2-methoxyethanol and 2-ethoxyethanol in shipbuilding facilities

(Sparer *et al.*, 1988; Welch and Cullen, 1988; Welch *et al.*, 1988). Exposure concentrations of at least 76 ppm 2-methoxyethanol for up to 112 weeks were reported in the 1938 study; these exposures resulted in bone marrow toxicity, anemia, and severe neurotoxicity. There was no evidence of 2-methoxyethanol toxicity in the 1982 study in which a closed system was used and the highest air concentration of 2-methoxyethanol was 20 ppm. In the 1986 study, dermal and inhalation exposures of up to 24 ppm 2-ethoxyethanol resulted in spermatotoxicity and urine concentrations of ethoxyacetic acid ranging from 16 to 163 mg/g creatinine. In the 1988 studies, the combined exposure to 2-methoxyethanol and 2-ethoxyethanol by inhalation at concentrations up to 5 ppm 2-methoxyethanol and 22 ppm 2-ethoxyethanol was confirmed by identification of specific alkoxyacetic acid metabolites in the urine. The toxic responses in these studies included lowered sperm counts and suggested that anemia and granulocytopenia could have been related to exposure.

REPRODUCTIVE TOXICITY

2-Methoxyethanol and 2-ethoxyethanol are both potent male reproductive toxicants in mice, rats, guinea pigs, rabbits, and dogs. In these animals, exposure to 2-methoxyethanol or 2-ethoxyethanol by the subcutaneous, dermal, oral, or inhalation route resulted in testicular atrophy and decreased fertility caused by spermatotoxicity (Stenger *et al.*, 1971; Nagano *et al.*, 1979; Foster *et al.*, 1983; Miller *et al.*, 1983a; Chapin *et al.*, 1985; Hobson *et al.*, 1986; Oudiz and Zenick, 1986). The most sensitive cells were shown to be the primary spermatocytes in the pachytene stage of meiosis and secondary spermatocytes (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987). In contrast, there is ample evidence from studies in mice and rats that administration of 2-butoxyethanol by the oral gavage or inhalation route has no effect on the male reproductive system (Nagano *et al.*, 1979; Doe, 1984a; Krasavage, 1986).

2-Methoxyethanol and, to a lesser extent, 2-ethoxyethanol are potent teratogens. Studies have been conducted in mice, rats, rabbits, and monkeys using dermal, oral, and inhalation routes of administration (Hardin *et al.*, 1981, 1982; Nagano *et al.*, 1981; Doe, 1984b; Hanley *et al.*, 1984; Horton *et al.*, 1985; Greene *et al.*, 1987; Scott *et al.*, 1989; Feuston *et al.*, 1990). For 2-methoxyethanol, a no-observed-effect level of 10 ppm was

established for pregnant mice, rats, and rabbits (Hanley *et al.*, 1984). Additionally, a no-observed-effect level of 100 mg/kg was established for pregnant mice when 2-methoxyethanol was administered in a single dose on Day 11 of gestation (Horton *et al.*, 1985). Adverse effects on maternal animals included prolonged gestation and reductions in body weight and weight gain. The toxicity of 2-butoxyethanol on the reproductive system of female F344 rats and CD-1 mice was limited to fetal mortality and decreased body weight and weight gain in the dams; these effects were noted only after administration of doses that caused death to 20% of the dams. There was no teratogenicity in offspring of dams that were dosed below the maternal LD_{20} (Schuler *et al.*, 1984; Tyl *et al.*, 1984;).

CARCINOGENICITY

There have been no adequate carcinogenicity studies conducted with any of the glycol alkyl ethers.

IMMUNOTOXICITY

The data from a cell-mediated immunity assay in mice suggested that 2-methoxyethanol and 2-ethoxyethanol might stimulate the immune system. Allogenic mice given L1210 leukemia cells and dosed with up to 100 mg/kg 2-methoxyethanol or 2400 mg/kg 2-ethoxyethanol 12 days before transplant survived, while those without chemical treatment developed leukemia and died (Houchens *et al.*, 1984). However, a second study in mice used 2-methoxyethanol at doses of up to 1000 mg/kg, and while thymic atrophy occurred, no changes in bone marrow cellularity, leukocyte counts, or immune function were observed (House *et al.*, 1985). Two studies of the potential effects of 2-methoxyethanol on immune function conducted in Sprague-Dawley and F344 rats yielded conflicting data. Exon *et al.* (1991) reported that natural killer cell cytotoxic responses were enhanced in male and female Sprague-Dawley rats administered doses of 1600 to 6000 ppm 2-methoxyethanol in drinking water for 21 days; however, delayed type hypersensitivity was suppressed as was gamma interferon production and interleukin-2 production by spleen cells. The authors suggested that 2-methoxyethanol exerted immunomodulatory effects in the rats. However, Smialowicz *et al.* (1991) reported variable responses to sheep red blood cell and trinitrophenyl-lipopolysaccharide antigens

(depending on dose and schedule of 2-methoxyethanol administration), no alterations in natural killer cell activity, mixed lymphocyte, cytotoxic T-cell, or lymphoproliferative responses, and a reduction in interleukin-2 production by spleen cells.

GENETIC TOXICITY

None of the 3 glycol ethers, 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol, was mutagenic in *Salmonella typhimurium*, with or without S9 activation (McGregor *et al.*, 1983; Shimizu *et al.*, 1985; Zeiger *et al.*, 1985, 1992). Additional genotoxicity data are available for the monomethyl and monoethyl ethers; most of the results were negative, but a few positive responses were reported for each chemical in tests for induction of chromosome damage in mammalian cells.

2-Methoxyethanol, tested as a vapor, did not induce mutations in the *Drosophila* sex-linked recessive lethal assay (McGregor *et al.*, 1983). In addition, it did not cause gene mutations in the yeast *Schizosaccharomyces pombe*, assayed either in a host-mediated assay (Barale *et al.*, 1979) or in culture, with or without S9 (Abbondandolo *et al.*, 1980). 2-Methoxyethanol did not produce unscheduled DNA synthesis in cultured human embryo fibroblasts, with or without S9 (McGregor *et al.*, 1983). It did, however, induce sperm abnormalities in mice (McGregor *et al.*, 1983; Anderson *et al.*, 1987) and in rats (Anderson *et al.*, 1987). No increase in dominant lethal mutations was observed in mice treated with 2-methoxyethanol (Anderson *et al.*, 1987); similar tests in rats yielded somewhat conflicting results. Some laboratories reported small, inconclusive effects in either CD (McGregor *et al.*, 1983) or F344 (Chapin *et al.*, 1985) rats, while other laboratories found no evidence of dominant lethal mutations in either CD (Anderson *et al.*, 1987) or Sprague-Dawley (Rao *et al.*, 1983) rats treated with 2-methoxyethanol. The severe effect of 2-methoxyethanol on male fertility confounded the interpretation of the dominant lethal data in the 2 studies that noted an increase in the number of post-implantation losses.

2-Ethoxyethanol was not mutagenic in *Escherichia coli* (Shimizu *et al.*, 1985), with or without S9, and it did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila* treated by feeding or by injection (Valencia *et al.*, 1985). No induction of gene mutations was noted in mouse lymphoma L5178Y cells (Myhr *et al.*, 1986) or Chinese

hamster ovary (CHO) cells (Guzzie *et al.*, 1986) after treatment with 2-ethoxyethanol. However, increased frequencies of both chromosomal aberrations and sister chromatid exchanges (SCEs) were observed in CHO cells treated with 2-ethoxyethanol in the absence of S9; SCE frequencies were also increased in these cells in the presence of S9 (Guzzie *et al.*, 1986; Galloway *et al.*, 1987). No increase in the number of micronucleated polychromatic erythrocytes was reported in peripheral blood samples of Swiss-Webster mice administered a single intraperitoneal injection of 2-ethoxyethanol at doses of 25% to 80% of the LD_{50} (2589 mg/kg) (Guzzie *et al.*, 1986).

Study Rationale and Design

2-Methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were nominated for testing by the United Auto Workers International Union, NIOSH, and the Consumer Product Safety Commission based on their current and increasing patterns of usage, their prevalence in waste sites, the gaps in toxicity data identified in a 1984 review of glycol ethers (Lucier and Hooks, 1984), and the concern about carcinogenic potential.

Since occupational exposure to glycol alkyl ethers would normally occur dermally or by inhalation, these were considered to be the most appropriate routes of administration. However, preliminary studies indicated that most of a dermal dose of the labile glycol alkyl ethers would evaporate if unoccluded. The difficulties and uncertainties of dose application in chronic dermal studies and concerns about the general applicability of the findings of such studies caused the abandonment of this route of application. Because preliminary disposition studies showed that maximum systemic exposure could be readily achieved by oral administration, dosed drinking water was used in the prechronic studies to determine the relative toxicity of the 3 glycol alkyl ethers and to subsequently select 1 of the 3 compounds for further investigation.

Thus, toxicity studies were conducted in male and female F344/N rats and B6C3F$_1$ mice by the drinking water route to compare the 2-week and 13-week general toxicity of the 3 ethylene glycol ethers, determine the necessity for conducting chronic carcinogenicity tests on all 3 compounds, and determine the appropriate doses for long-term tests in the event that they are required; the genotoxicity of the 3 glycol alkyl ethers was also assessed

during these studies. The data from additional stop-exposure and leukemia inhibition studies of the glycol alkyl ethers in male F344/N rats are also included in this report.

MATERIALS AND METHODS

Procurement and Characterization of Ethylene Glycol Ethers

2-Methoxyethanol (CAS Number 109-86-4) and 2-ethoxyethanol (CAS Number 110-80-5) were obtained from Kodak Laboratory Chemicals (Rochester, NY). 2-Butoxyethanol (CAS Number 111-76-2) was obtained from Aldrich Chemical Company (Milwaukee, WI). Lot E16 of 2-methoxyethanol, Lot D16 of 2-ethoxyethanol, and Lot BT00504LP of 2-butoxyethanol were used in the 2-week and 13-week studies in rats and mice and in the stop-exposure studies in male rats.

Identity and purity analyses were conducted on all 3 isomers at Midwest Research Institute (MRI, Kansas City, MO). The clear, colorless liquids were identified as either 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The spectra were consistent with the structures of the chemicals, with available literature references (*Sadtler Standard Spectra*) and with previous analyses of 2-ethoxyethanol and 2-butoxyethanol performed at MRI. Ultraviolet/visible spectroscopy for 2-butoxyethanol gave a spectrum consistent with the structure. Elemental analyses of 2-methoxyethanol and 2-ethoxyethanol for carbon and hydrogen agreed with theoretical values. Elemental analysis of 2-butoxyethanol for hydrogen was slightly low; analysis for carbon was in agreement with theoretical values. Karl Fischer water analysis indicated the presence of 0.080% ± 0.015% water for 2-methoxyethanol, 0.051% ± 0.007% water for 2-ethoxyethanol, and 0.079% ± 0.009% water for 2-butoxyethanol. Potentiometric titration indicated less than 0.005 mEq acid/g sample for both 2-methoxyethanol and 2-ethoxyethanol. Potentiometric titration of 2-butoxyethanol indicated 0.0011 ± 0.0001 mEq acid/g of compound. Functional group (hydroxyl) titration indicated a purity of 98.3% ± 0.05% for 2-methoxyethanol and 101.1% ± 0.5% for 2-butoxyethanol. Oxidation/reduction titration of 2-ethoxyethanol indicated a purity of 100.3% ± 0.7%. Gas chromatography utilizing 2 separate systems indicated a purity of 100.2% ± 1.2% for 2-methoxyethanol, 100.5% ± 0.7% for 2-ethoxyethanol, and 100.5% ± 0.3% for 2-butoxyethanol relative to frozen reference standards. Thin-layer chromatography of 2-butoxyethanol utilizing 2 systems indicated

no impurities in the chemical used for the 13-week and stop-exposure studies. Overall purity was approximately 98% for 2-methoxyethanol and approximately 99% for 2-ethoxyethanol and 2-butoxyethanol.

Subsequent reanalyses of the bulk compounds were performed at EG&G Mason Research Institute. Results of analyses for peroxide content and by functional group titration (2-methoxyethanol and 2-butoxyethanol) or oxidation/reduction titration (2-ethoxyethanol) indicated that the purity of the chemicals relative to the reference standards remained unchanged throughout the studies.

Dose Formulations

Dose formulations were prepared by mixing the appropriate amount of each isomer with deionized water (w/w) to achieve the desired concentrations. Dose formulations for the studies were prepared as needed and were used within 3 weeks of preparation.

Stability studies conducted by MRI on dose formulations indicated that doses of 20,000 ppm 2-methoxyethanol and 10,000 ppm 2-butoxyethanol were stable for up to 3 weeks when stored in the dark at 5°C in sealed glass containers. 2-Methoxyethanol- or 2-butoxyethanol-dosed water stored in rodent drinking bottles was also found to be stable for at least 4 days. Dose formulations of 4000 ppm 2-ethoxyethanol were found to be stable for 3 weeks in the dark at room temperature in sealed glass containers. Dose formulations for all studies were stored in the dark at 4° ± 3°C. Results of all dose formulation analyses were within 10% of theoretical concentrations with 1 exception that led to a dosing error. The 3000 ppm stop-exposure study dose of 2-methoxyethanol mixed on 23 August 1988 was analyzed on 6 September 1988 and found to contain 5820 ppm 2-methoxyethanol. All cages in this dose group were presumed to have been misdosed for 3 days.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice used in these studies were obtained from Taconic Farms (Germantown, NY). Rats and mice were shipped to the study laboratory at approximately 4 to 5 weeks of age, quarantined for 1 to 2 weeks, and then placed on study at about 5 to 7 weeks of age. Rats for the 13-week base studies were received in 2 shipments (1 for the base studies and 1 for the clinical pathology studies). In the 2-week studies, 2 animals per species per sex were examined for disease and parasites; no abnormalities were found. Blood samples were collected from the retroorbital sinuses of 5 animals per sex for each isomer and analyzed for viral antibody titers. For the 2-methoxyethanol and 2-butoxyethanol studies, blood samples were collected when animals were released from quarantine; for the 2-ethoxyethanol study, blood samples were collected at study termination. No positive antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b) were found for animals in any of the 13-week studies. Additional details concerning study design and performance are listed in Table 2.

Rats were housed 5 animals per cage and mice were housed individually for the 2-week and 13-week studies. Animal rooms were maintained at 60° to 77°F and 20% to 70% relative humidity with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day. Feed and drinking water solutions were available *ad libitum*.

In the 2-week studies of each isomer, groups of 5 rats and 5 mice per sex per dose group were administered the study chemical in drinking water available *ad libitum*. Target dose levels selected for rats and mice receiving 2-methoxyethanol were 0, 200, 400, 600, 1000, or 1200 mg/kg body weight. Target dose levels for rats and mice receiving 2-ethoxyethanol were 0, 300, 600, 900, 1500, or 2500 mg/kg body weight. Target dose levels for rats and mice receiving 2-butoxyethanol were 0, 100, 150, 250, 400, or 650 mg/kg body weight. Sufficient chemical was added to the drinking water solutions to achieve these doses based on historical water consumption data.

Dose selections for each 13-week study were based on the results of the respective 2-week studies. Due to a dose-related decrease in water consumption, the test articles were

administered at a constant concentration (ppm) in the 13-week studies rather than on a mg/kg body weight basis as in the 2-week studies. In the 13-week studies of each isomer, 10 rats and 10 mice per sex per dose group were administered test articles in drinking water. In the 2-methoxyethanol studies, rats received 0, 750, 1500, 3000, 4500, or 6000 ppm and mice received 0, 2000, 4000, 6000, 8000, or 10,000 ppm. In the 2-ethoxyethanol studies, rats received 0, 1250, 2500, 5000, 10,000, or 20,000 ppm and mice received 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. In the 2-butoxyethanol studies, rats and mice received 0, 750, 1500, 3000, 4500, or 6000 ppm. Drinking water was available *ad libitum* for 13 weeks.

Complete necropsies were performed on all base-study animals in the 2-week and 13-week studies. The following organs from rats and mice were weighed: heart, right kidney, liver, lung, thymus, and right testis. Organs and tissues were examined for gross lesions and were fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

For animals in the 2-week studies, complete histopathologic examinations were performed only on those organs showing gross evidence of lesions. For animals in the 13-week studies, complete histopathologic examinations of protocol-required tissues were performed on all control animals, all animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all animals in higher dose groups inclusive of early deaths and survivors. Gross lesions and selected tissues were examined in the lower dose groups to no-observed-effect level. Tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

In the 13-week studies of ethylene glycol ethers, hematology and clinical chemistry evaluations were performed on supplemental rats (10 males and 10 females per dose group per test article) at Weeks 1 and 3 and on base-study rats at study termination (Week 13). Urine samples were collected from base-study rats for evaluation at the end of the study. Animals were administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in drinking water available *ad libitum*. Dose levels were 0, 750, 1500, 3000, 4500, or 6000 ppm for rats receiving 2-methoxyethanol or 2-butoxyethanol and 0, 1250, 2500, 5000, 10,000, or 20,000 ppm for rats receiving 2-ethoxyethanol.

At all time points, rats were anesthetized with 70% CO_2:30% O_2, and blood samples were collected from the retroorbital sinus using capillary tubes. Blood samples were placed in EDTA tubes for hematologic analyses and in plain tubes devoid of an anticoagulant for clinical chemistry analyses. After blood samples were collected, bone marrow cells were collected from the right femur of rats for determination of total nucleated cell counts (Thompson *et al.*, 1991). On Day 90, rats were placed individually in metabolism cages for the collection of 16-hour urine samples. During this period, animals had access to feed but not water. Samples were collected in tubes that were immersed in an ice/water bath.

Hematologic determinations were performed with a Series 7000 cell counter and a Series 810 whole blood platelet analyzer (Baker Instruments, Allentown, PA). Reticulocyte counts were determined by microscopic examination of blood smears that had been incubated with new methylene blue. Leukocyte differentials were calculated from percentages of cell types determined from microscopic examination of Wright's-stained blood smears. Methemoglobin concentrations were measured using a spectrophotometric method (Evelyn and Malloy, 1938). Clinical chemistry variables were measured with a Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). Clinical pathology variables evaluated in the 13-week study are listed in Table 2.

Sperm Morphology and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm morphology evaluations were performed on rats (10 animals per sex per dose group) and mice (10 animals per sex per dose group) from the 13-week studies. Male rats receiving 2-methoxyethanol at dose levels of 0, 750, 1500, or 3000 ppm and female rats receiving 2-methoxyethanol at dose levels of 0, 1500, 3000, or 4500 ppm were evaluated. Male mice receiving 0, 2000, 4000, or 6000 ppm 2-methoxyethanol and female mice receiving 0, 6000, 8000, or 10,000 ppm 2-methoxyethanol were evaluated. Rats administered 2-ethoxyethanol at dose levels of 0, 2500, 5000, or 10,000 ppm and mice administered 2-ethoxyethanol at dose levels of 0, 5000, 10,000, or 20,000 ppm were evaluated. Also, rats and mice administered 0, 3000, 4500, or 6000 ppm 2-butoxyethanol were evaluated. Methods were those described by Morrissey *et al.* (1988). Briefly, for the 7 days prior to sacrifice, the vaginal vaults of 10 females of each species per dose group were lavaged and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm morphology was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the epididymal tail. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for 5 fields per slide.

Following completion of sperm motility estimates, each cauda epididymis was placed in buffered saline solution (0.9%). Cauda were gently minced and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were enumerated using a hemacytometer.

Stop-Exposure Studies in Male Rats

Dose selections for the stop-exposure studies were based on the results of the 2-week studies of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. In each stop-exposure study, 30 male rats per dose group were administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in drinking water. Dose levels for rats receiving 2-methoxyethanol were 0, 1500, 3000, or 6000 ppm (Note: During Week 5 of the stop-exposure study of 2-methoxyethanol, rats in the 3000 ppm dose group received 5820 ppm 2-methoxyethanol for approximately 3 days). Dose levels for rats receiving 2-ethoxyethanol were 0, 5000, 10,000, or 20,000 ppm. Dose levels for rats receiving 2-butoxyethanol were 0, 1500, 3000, or 6000 ppm (Note: During Week 6 of the stop-exposure study of 2-butoxyethanol, rats in the 1500 ppm dose group received 2500 ppm 2-ethoxyethanol). Test articles were administered daily for 60 days in drinking water that was available *ad libitum*. At the end of the treatment period, 10 rats per dose group were killed, except in the case of early deaths. If lesions were found at the 60-day necropsy, half of the remaining animals were killed after a 30-day recovery period, and the other half were killed after a 56-day recovery period. Animals were housed 5 per cage in the same room as the animals in the 13-week studies. At necropsy, the right and left testes were removed and weighed. The testes and the caput and cauda of the left epididymis were examined microscopically. Organs for rats in the 30- and 56-day recovery groups in the 2-butoxyethanol stop-exposure study were not processed for histology because no microscopic lesions attributable to chemical exposure were found after the 60-day exposure period.

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers

EXPERIMENTAL DESIGN	
Study Laboratory	EG&G Mason Research Institute (Worcester, MA)
Size of Study Groups	2-Week Studies: 5 males and 5 females per species per dose group 13-Week Studies: Base Studies: 10 males and 10 females per species per dose group Clinical Pathology Study: 20 male and 20 female rats per dose group Stop-Exposure Studies: 30 male rats per dose group
Route of Administration	Drinking water
Doses/Duration of Dosing	2-Week Studies: *2-Methoxyethanol*: Rats and mice: 0, 200, 400, 600, 1000, or 1200 mg/kg daily for 14 days *2-Ethoxyethanol*: Rats and mice: 0, 300, 600, 900, 1500, or 2500 mg/kg daily for 14 days *2-Butoxyethanol*: Rats and mice: 0, 100, 150, 250, 400, or 650 mg/kg daily for 14 days 13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 0, 750, 1500, 3000, 4500, or 6000 ppm daily for 13 weeks Mice: 0; 2000; 4000; 6000; 8000; or 10,000 ppm daily for 13 weeks *2-Ethoxyethanol*: Rats: 0; 1250; 2500; 5000; 10,000; or 20,000 ppm daily for 13 weeks Mice: 0; 2500; 5000; 10,000; 20,000; or 40,000 ppm daily for 13 weeks *2-Butoxyethanol*: Rats and mice: 0, 750, 1500, 3000, 4500, or 6000 ppm daily for 13 weeks Clinical Pathology Studies: Same as 13-week base studies; daily for 21 days Stop-Exposure Studies: *2-Methoxyethanol*: 0, 1500, 3000, or 6000 ppm; daily for 60 days *2-Ethoxyethanol*: 0; 5000; 10,000; 20,000 ppm; daily for 60 days *2-Butoxyethanol*: 0, 1500, 3000, or 6000 ppm; daily for 60 days
Date of First Dose	2-Week Studies: *2-Methoxyethanol*: Rats: 21 March 1988 (males), 22 March 1988 (females) Mice: 23 March 1988 (males), 24 March 1988 (females) *2-Ethoxyethanol*: Rats: 18 January 1988 (males), 19 January 1988 (females) Mice: 20 January 1988 (males), 21 January 1988 (females) *2-Butoxyethanol*: Rats: 22 February 1988 (males), 23 February 1988 (females) Mice: 24 February 1988 (males), 25 February 1988 (females) 13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 19 July 1988 (males), 21 July 1988 (females) Mice: 12 July 1988 (males), 14 July 1988 (females) *2-Ethoxyethanol*: Rats: 3 May 1988 (males), 5 May 1988 (females) Mice: 26 April 1988 (males), 28 April 1988 (females) *2-Butoxyethanol*: Rats: 14 June 1988 (males), 16 June 1988 (females) Mice: 21 June 1988 (males), 23 June 1988 (females)

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Date of First Dose (continued)	13-Week Studies (continued): Clinical Pathology Studies: *2-Methoxyethanol*: 5 October 1988 (males), 6 October 1988 (females) *2-Ethoxyethanol*: 4 August 1988 (males), 5 August 1988 (females) *2-Butoxyethanol*: 31 August or 1 September 1988 (males), 1 or 2 September 1988 (females) Stop-Exposure Studies: *2-Methoxyethanol*: 22 July 1988 *2-Ethoxyethanol*: 6 May 1988 *2-Butoxyethanol*: 17 June 1988
Date of Last Dose	2-Week Studies: *2-Methoxyethanol*: Rats: 4 April 1988 (males), 5 April 1988 (females) Mice: 6 April 1988 (males), 7 April 1988 (females) *2-Ethoxyethanol*: Rats: 1 February 1988 (males), 2 February 1988 (females) Mice: 3 February 1988 (males), 4 February 1988 (females) *2-Butoxyethanol*: Rats: 7 March 1988 (males), 8 March 1988 (females) Mice: 9 March 1988 (males), 10 March 1988 (females) 13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 18-19 October 1988 (males), 20-21 October 1988 (females) Mice: 11-12 October 1988 (males), 13-14 October 1988 (females) *2-Ethoxyethanol*: Rats: 2-3 August 1988 (males), 4-5 August 1988 (females) Mice: 26-27 July 1988 (males), 28-29 July 1988 (females) *2-Butoxyethanol*: Rats: 13-14 September 1988 (males), 15-16 September 1988 (females) Mice: 20-21 September 1988 (males), 22-23 September 1988 (females) Clinical Pathology Studies: *2-Methoxyethanol*: 10 or 26 October 1988 (males), 11 or 27 October 1988 (females) *2-Ethoxyethanol*: 9 or 25 August 1988 (males), 10 or 26 August 1988 (females) *2-Butoxyethanol*: 6 or 21 September 1988 (males), 7 or 22 September 1988 (females) Stop-Exposure Studies: *2-Methoxyethanol*: 20 September 1988 *2-Ethoxyethanol*: 5 July 1988 *2-Butoxyethanol*: 16 August 1988
Necropsy Dates	2-Week Studies: *2-Methoxyethanol*: Rats: 4 April 1988 (males), 5 April 1988 (females) Mice: 6 April 1988 (males), 7 April 1988 (females) *2-Ethoxyethanol*: Rats: 1 February 1988 (males), 2 February 1988 (females) Mice: 3 February 1988 (males), 4 February 1988 (females) *2-Butoxyethanol*: Rats: 7 March 1988 (males), 8 March 1988 (females) Mice: 9 March 1988 (males), 10 March 1988 (females)

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Necropsy Dates (continued)	13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 18-19 October 1988 (males), 20-21 October 1988 (females) Mice: 11-12 October 1988 (males), 13-14 October 1988 (females) *2-Ethoxyethanol*: Rats: 2-3 August 1988 (males), 4-5 August 1988 (females) Mice: 26-27 July 1988 (males), 28-29 July 1988 (females) *2-Butoxyethanol*: Rats: 13-14 September 1988 (males), 15-16 September 1988 (females) Mice: 20-21 September 1988 (males), 22-23 September 1988 (females) Stop-Exposure Studies: *2-Methoxyethanol*: 20 September, 20 October, or 15 November 1988 *2-Ethoxyethanol*: 5 July, 4 August, or 30 August 1988 *2-Butoxyethanol*: 16 August or 15 September 1988
Type and Frequency of Observation	2-Week Studies: Animals were observed twice daily and were weighed at the start of the studies, at the end of Week 1, and at necropsy. Clinical observations were recorded daily. Water consumption by cage was measured 2 times per week. 13-Week Studies: Base Studies: Animals were observed twice daily and were weighed at the start of the studies, weekly thereafter, and at necropsy. Clinical observations were recorded weekly. Water consumption by cage was measured 2 times per week. Clinical Pathology Studies: Animals were observed twice daily. Stop-Exposure Studies: Same as 13-week base studies.
Necropsy and Histologic Examinations	2-Week and 13-Week Base Studies: Complete necropsies were performed on all animals in the base studies. The protocol for the 2-week studies required that only organs showing evidence of gross lesions be examined microscopically. The protocol for the 13-week studies required that tissues be examined microscopically in all control animals, all animals in the highest dose group with at least 60% survivors, and all animals in the higher dose groups (inclusive of early deaths and survivors). These tissues included: adrenal glands, bone (femur) with marrow, brain (3 sections), esophagus, eyes, gallbladder (mice), gross lesions, heart/aorta, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, larynx, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pituitary gland, pharynx, preputial or clitoral glands, prostate gland, salivary glands, seminal vesicles, skin, spinal cord/sciatic nerve, spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, and vagina (SMVCE animals only).

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Necropsy and Histologic Examinations (continued)	Tissues examined in the lower dose groups in the 2-week studies in rats were the testis and epididymis. In the 2-week studies in mice, no tissues were designated for examination in the lower dose groups. In the 13-week studies of 2-methoxyethanol and 2-ethoxyethanol in rats, tissues examined in the lower dose groups were bone (2-methoxyethanol), bone marrow, the epididymis (2-ethoxyethanol), liver, ovary, preputial or clitoral gland, prostate gland, seminal vesicle, spleen, stomach, testis (2-ethoxyethanol), thymus, uterus, and vagina (2-ethoxyethanol). In the 13-week study of 2-butoxyethanol in rats, bone marrow and the epididymis, liver, spleen, testis, and uterus were examined in the lower dose groups. Tissues examined for mice in the lower dose groups in the 13-week studies of 2-methoxyethanol and 2-ethoxyethanol were the adrenal gland (females), ovary (2-methoxyethanol), spleen, testis, thymus (2-methoxyethanol), and uterus (2-methoxyethanol). In the 13-week study of 2-butoxyethanol in mice, no tissues were designated for examination in the lower dose groups. Stop-Exposure Studies: Tissues examined microscopically were the right and left testes and caput and cauda of the left epididymis. Organs weighed were the right epididymis and right testis.
Supplemental Evaluations	Clinical Pathology Studies: 13-Week Base Studies: On Days 5 and 21, blood samples were collected from the retroorbital sinuses of rats designated for the clinical pathology studies. Week 13 analyses were conducted on samples obtained from rats in the base studies. Urinalysis was done on Week 13 samples collected overnight from the base study animals. Hematology parameters evaluated included hematocrit (HCT), hemoglobin (HGB), erythrocytes (RBCs), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, reticulocytes, leukocyte count and differential, nucleated erythrocytes, methemoglobin, and total bone marrow cellularity. Clinical chemistry parameters evaluated included urea nitrogen (UN), creatinine, total protein, albumin, alkaline phosphatase (AP), alanine aminotransferase (ALT), creating kinase, and bile acids. Urinalysis parameters were urine volume, specific gravity, and urine pH. Sperm Morphology and Vaginal Cytology Evaluations (13-Week Base Studies): Males were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages. Animals in the following dose groups were evaluated. *2-Methoxyethanol:* Rats: Males, 0, 750, 1500, or 3000 ppm (10 animals per dose group), Females, 0, 1500, 3000, or 4500 ppm (10 animals per dose group) Mice: Males, 0, 2000, 4000, or 6000 ppm (10 animals per dose group), Females, 0; 6000; 8000; or 10,000 ppm (10 animals per dose group) *2-Ethoxyethanol:* Rats: 0, 2500, 5000, or 10,000 ppm (10 animals per sex per dose group) Mice: 0, 5000, 10,000, or 20,000 ppm (10 animals per sex per dose group) *2-Butoxyethanol:* Rats and mice: 0, 3000, 4500, or 6000 ppm (10 animals per sex per dose group)

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N Rats B6C3F, Mice
Animal Source	Taconic Farms (Germantown, NY)
Time Held Before Study	2-Week Studies: Rats: 1½ weeks Mice: 2 weeks 13-Week Studies: *2-Methoxyethanol* and *2-Butoxyethanol*: approximately 2 weeks *2-Ethoxyethanol*: Rats, approximately 2 weeks; Mice, approximately 1 week Stop-Exposure Studies: 2 weeks
Age When Placed on Study	2-Week Studies: 6-7 weeks 13-Week Studies: Base Studies: 5-6 weeks Clinical Pathology Studies: *2-Methoxyethanol* and *2-Butoxyethanol*: approximately 7 weeks *2-Ethoxyethanol*: 19 weeks Stop-Exposure Studies: Approximately 6 weeks
Age When Killed	2-Week Studies: *2-Methoxyethanol*: 9 weeks *2-Ethoxyethanol* and *2-Butoxyethanol*: 8 weeks 13-Week Studies: Base Studies: 18-19 weeks Stop-Exposure Studies: 15, 19, or 21 weeks
Method of Animal Distribution	Animals were weighed and were randomized using a computer program.
Diet	NIH-07 Open Formula Pellets (Zeigler Brothers, Inc., Gardners, PA) and deionized water (filtered and untreated) available *ad libitum*
Animal Room Environment	Rats were housed 5 animals per cage and mice housed individually for all base studies. Temperature was maintained at 60°-77°F and relative humidity at 20%-70%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicology Studies

MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

Testing of 2-ethoxyethanol was performed as reported by Zeiger *et al.* (1985), and testing of 2-methoxyethanol and 2-butoxyethanol was performed as reported by Zeiger *et al.* (1992). The chemicals were sent to the testing laboratories as a coded aliquots. They were incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, TA1537, and TA97) either in buffer or S9 mix (metabolic activation enzymes and cofactors from

Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted for 2 days following incubation at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least 5 doses of the test chemical. High dose was limited by experimental design to 10,000 µg/plate. Varied concentrations of S9 were used in the tests with 2-methoxyethanol and 2-butoxyethanol.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr *et al.* (1985). 2-Ethoxyethanol was supplied as a coded aliquot. The high dose of 2-ethoxyethanol was limited by experimental design to 5 µL/mL. Mouse lymphoma L5178Y cells were maintained at 37°C as suspension cultures in Fischer's medium supplemented with *l*-glutamine, sodium pyruvate, pluronic F68, antibiotics, and heat-inactivated horse serum; normal cell cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, glycine) for 1 day, to medium containing THG for 1 day, and to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with 2-ethoxyethanol continued for 4 hours, at which time the medium plus chemical was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48 hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant cells ($TK^{-/-}$); 600 cells were plated in nonselective

medium and soft agar to determine cloning efficiency. Plates were incubated at 37°C in 5% CO_2 for 10 to 12 days. This assay was initially performed without S9; if a clearly positive response was not obtained, the experiment was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced Fischer 344 male rats.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1987). 2-Ethoxyethanol and 2-butoxyethanol were sent to the laboratory as coded aliquots. They were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least 3 doses of the particular test chemical. In the SCE test, the highest testable dose of 2-butoxyethanol, in the absence of S9, was limited by toxicity to 3000 (Trial 1) and 3500 µg/mL (Trial 2); with S9, no toxicity was observed and the high dose was limited to 5000 µg/mL. In the Abs test with 2-butoxyethanol, high dose was limited to 5000 µg/mL. For 2-ethoxyethanol, the high dose was not limited by excessive toxicity or lack of solubility and reached 9510 µg/mL in both the SCE and Abs tests. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

In the standard SCE test without S9, CHO cells were incubated for approximately 26 hours with test chemical in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After about 26 hours (25.5 hours for 2-ethoxyethanol), the medium containing test chemical was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the test chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no test chemical and incubation proceeded for an additional 26 hours (25.5 hours for 2-ethoxyethanol), with Colcemid present for the final 2 hours. Harvesting and staining

were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen with 2-butoxyethanol in the absence of S9, incubation time was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with the test chemical for 8.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with the test chemical and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 8.5 to 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: because cell cycle delay was anticipated for 2-butoxyethanol in the absence of S9, the incubation period was extended in 2 of the 3 trials.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One/two hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

DROSOPHILA MELANOGASTER SEX-LINKED RECESSIVE LETHAL TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described in Valencia *et al.* (1985) and Mason *et al.* (1992). 2-Ethoxyethanol was supplied as a coded aliquot. It was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, it was retested by injection into adult males.

To administer a chemical by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivers a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of 2-ethoxyethanol at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of 2-ethoxyethanol in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of 2-ethoxyethanol dissolved in saline and allowed to recover for 24 hours. Treated males were mated to 3 *Basc* females for 3 days and given fresh females at 2-day intervals to produce 3 matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier post-meiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). A cluster was identified in the feeding experiment in test 2 and all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight

data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any 1 strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in

revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE LYMPHOMA MUTAGENICITY DATA

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for both trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for a chemical to be considered capable of inducing TFT resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

ANALYSIS OF CHO CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.*, 1985). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at 1 dose point is less than 0.01; the probability for such a chance occurrence at 2 dose points is less than 0.001. An increase of 20% or greater at any single dose, along with a trend P-value less than 0.025, was considered weak evidence of activity; increases at 2 or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal (Galloway *et al.*, 1985).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1985). For a single trial, a statistically significant ($P<0.05$) difference for 1 dose point and a significant trend ($P<0.005$) were considered weak evidence for a positive response; significant differences for 2 or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any 1 dose point, led to a conclusion of equivocal activity.

ANALYSIS OF *DROSOPHILA MELANOGASTER* DATA

Sex-linked recessive lethal data were analyzed by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P-value was less than or equal to 0.01 and the mutation frequency in the tested group was greater that 0.10%, or if the P-value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P-value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P-value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P-value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Quality Assurance

The animal studies of the ethylene glycol ethers were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of EG&G Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Studies in F344/N Rats

No rats in the 2-week studies of 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol died or were killed before the end of the study (Table 3). The mean final body weights and mean body weight changes for females receiving 400 mg/kg 2-methoxyethanol and for males and females receiving 600, 1000, or 1200 mg/kg 2-methoxyethanol were notably lower than those for the control group. In the 2-ethoxyethanol study, mean final body weights and mean body weight changes for male rats in all treated groups were notably lower than those for the control group. For female rats receiving 1500 or 2500 mg/kg 2-ethoxyethanol for 2 weeks, mean final body weights and mean body weight changes were also notably lower than values for the control group. In the 2-butoxyethanol study, there were no marked differences from controls in mean body weight or body weight gain for treated male rats. For females receiving 150 or 400 mg/kg 2-butoxyethanol, mean weight gains were notably higher than those for the control group. However, the mean final body weight for females receiving 650 mg/kg 2-butoxyethanol was notably lower than the control value.

In the 2-week studies of ethylene glycol ethers, dose-related decreases in mean water consumption were noted for male and female rats treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol (Table 3). Average compound consumption increased in a dose-related manner for male and female rats treated with the ethylene glycol ethers. However, because of reduced water consumption, doses were below targeted levels for males and females treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol.

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
2-Methoxyethanol							
0	5/5	167	204	37		23.2	
200	5/5	167	212	45	104	20.4	116
400	5/5	168	201	34	99	17.7	206
600	5/5	168	180	12	88	14.7	273
1000	5/5	172	148	-24	73	11.6	393
1200	5/5	170	135	-35	66	9.9	418
2-Ethoxyethanol							
0	5/5	107	167	60		17.4	
300	5/5	110	152	42	91	16.5	200
600	5/5	108	148	40	89	14.9	357
900	5/5	108	156	48	93	16.2	572
1500	5/5	107	159	52	95	15.8	919
2500	5/5	107	135	28	81	13.7	1582
2-Butoxyethanol							
0	5/5	105	169	64		19.1	
100	5/5	108	171	63	101	18.6	73
150	5/5	108	167	59	99	18.3	108
250	5/5	108	175	67	104	18.1	174
400	5/5	107	178	70	105	16.4	242
650	5/5	108	173	65	102	13.8	346

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (mg/kg)	Survival	Mean Body Weight (grams) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
FEMALE							
2-Methoxyethanol							
0	5/5	133	156	23		18.6	
200	5/5	132	150	18	96	14.8	113
400	5/5	132	147	15	94	11.3	175
600	5/5	132	130	-2	83	9.4	231
1000	5/5	133	110	-23	71	6.7	297
1200	5/5	134	111	-23	71	6.2	326
2-Ethoxyethanol							
0	5/5	108	139	31		18.1	
300	5/5	109	135	27	97	15.7	192
600	5/5	109	130	21	94	14.5	360
900	5/5	109	136	27	98	14.8	526
1500	5/5	112	130	18	94	13.6	824
2500	5/5	111	115	4	82	11.8	1281
2-Butoxyethanol							
0	5/5	95	129	34		15.3	
100	5/5	93	133	40	103	15.9	77
150	5/5	93	137	44	106	14.5	102
250	5/5	93	135	41	104	12.8	152
400	5/5	93	136	43	105	11.1	203
650	5/5	92	116	23	89	7.8	265

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) x 100.

Dehydration, abnormal posture, and thin appearance were noted for males treated with 1000 or 1200 mg/kg 2-methoxyethanol. All females receiving 600, 1000, or 1200 mg/kg 2-methoxyethanol were dehydrated. Abnormal posture and a thin appearance were observed for all females in the 1000 and 1200 mg/kg groups, and all females receiving 1200 mg/kg were emaciated by the end of the study. No clinical signs of toxicity were observed for males or females treated with 2-ethoxyethanol or 2-butoxyethanol.

2-Methoxyethanol: In the 2-week study of 2-methoxyethanol, most changes in absolute and relative organ weights for rats were related to low final body weights, excluding changes in thymus and testis weights. Absolute and relative thymus weights decreased in a dose-related fashion for males and females as did absolute and relative testis weights for males.

In the 2-methoxyethanol study, chemical-related gross lesions were present only in rats from the 1000 and 1200 mg/kg groups. Gross lesions were observed in the forestomach and mesenteric lymph nodes of male and female rats in the 1200 mg/kg groups and female rats in the 1000 mg/kg group. Microscopic changes in the forestomach that corresponded to the gross lesions included hemorrhage and edema of the mucosa and focal necrosis and ulceration of the squamous epithelium. Mild hyperplasia of the forestomach squamous mucosa was also present and was generally associated with the focal areas of necrosis or ulceration. Sinusoidal congestion, hemorrhage, and erythrophagocytosis were present in the mesenteric lymph nodes, which appeared enlarged or reddened at necropsy. In addition to chemical-related gross lesions, the testis and epididymis from all dosed and control rats were examined microscopically. Degeneration was present in the testis of all male rats in the 400 to 1200 mg/kg groups. This degeneration consisted of moderate to marked loss of germinal epithelium and the presence of multinucleated spermatid giant cells and cell debris in the lumen of seminiferous tubules. In male rats in the 600, 1000, and 1200 mg/kg groups, the lumen of the epididymis contained necrotic cells and cell debris and only a few spermatozoa. Degeneration was of mild severity at the 400 mg/kg dose level, and in 1/5 rats administered 200 mg/kg 2-methoxyethanol, there was minimal degeneration of the testes.

2-Ethoxyethanol: Excluding changes in thymus and testis weights, the majority of changes in absolute and relative organ weights for rats in the 2-week study of 2-ethoxyethanol were related to the low final body weights. Dose-related decreases were noted for the absolute and relative thymus weights of males and females and the absolute and relative testis weights of males.

There were no chemical-related gross lesions in male or female rats in the 2-week study of 2-ethoxyethanol. At the end of the study, the testis and epididymis from all male rats were evaluated microscopically. Degeneration of the seminiferous tubules was present at concentrations of 1500 and 2500 mg/kg 2-ethoxyethanol. Morphologic features of testicular degeneration were similar to those described for the 2-methoxyethanol study. At the highest dose (2500 mg/kg), the severity of degeneration ranged from moderate to marked; in the 1500 mg/kg group, the severity ranged from minimal to mild. No testicular effects were seen in animals administered 2-ethoxyethanol in the drinking water at a concentration of 900 mg/kg or less.

2-Butoxyethanol: Changes in organ weights were minimal in the 2-week study of 2-butoxyethanol in rats, and, excluding a slight decrease in absolute and relative thymus weights for high-dose (650 mg/kg) female rats, most of these changes could be attributed to low final mean body weights. In the 2-week study of 2-butoxyethanol, there were no chemical-related gross lesions in male or female rats. Microscopic examination was limited to the testis and epididymis of dosed and control rats; there were no chemical-related microscopic lesions.

For the 13-week studies of the ethylene glycol ethers in rats, chemical administration was changed from a mg/kg basis to a constant ppm in the drinking water. The maximum concentrations used for 2-ethoxyethanol and 2-butoxyethanol were somewhat higher than the doses that were found to affect water consumption and cause minimal toxicity in the 2-week studies. For 2-methoxyethanol, the highest doses chosen (4500 and 6000 ppm) were too high when considering the marked body weight effects seen in the 2-week studies. A high dose of 3000 ppm would have been more appropriate.

13-Week Studies in F344/N Rats

In the 2-methoxyethanol study in rats, 8 males and 5 females in the 4500 ppm groups and all males and females in the 6000 ppm groups died or were killed prior to scheduled termination (Table 4). In the 2-ethoxyethanol study, 5 males and 7 females in the 20,000 ppm groups died or were killed early; due to the high mortality at this exposure level, the remaining male and female rats in the 20,000 ppm groups were removed from treatment during Week 9 of the study. No rats treated with 2-butoxyethanol died or were killed before the end of the 13-week study.

The final mean body weights for males and females receiving 1500 to 4500 ppm 2-methoxyethanol were notably lower than values for the control group. Body weight analyses were not performed for male or female rats in the 6000 ppm groups due to 100% mortality. In the 13-week study of 2-ethoxyethanol, males dosed with 10,000 or 20,000 ppm and females dosed with 5000 to 20,000 ppm had notably decreased final mean body weights when compared to the control group values. Mean body weight gains for males and females receiving 5000 to 20,000 ppm 2-ethoxyethanol were also notably lower than those of the control groups (Figures 1-3). The mean final body weights and mean weight gains for male and female rats treated with 4500 or 6000 ppm 2-butoxyethanol were notably less than the control values.

In the 13-week study of 2-methoxyethanol, decreases in mean water consumption were noted for males and females in the 3000 and 6000 ppm groups as well as for females in the 1500 ppm group (Table 4). For male and female rats treated with 2-ethoxyethanol or 2-butoxyethanol in the drinking water, average daily water consumption decreased, with a dose-related decrease occurring in females administered 2-butoxyethanol.

Average compound consumption increased in a dose-related manner for male and female rats treated with the ethylene glycol ethers for 13 weeks. However, in rats treated with 2-butoxyethanol, compound consumption generally decreased over the course of the study because of a decrease in water consumption.

TABLE 4 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
MALE							
2-Methoxyethanol							
0	10/10	129	311	182		21.2	
750	10/10	132	294	163	95	20.8	71
1500	10/10	127	259	132	81	21.4	165
3000	10/10	132	218	86	70	18.9	324
4500	2/10[4]	130	136	16	44	21.5	715
6000	0/10[4]	124	—	—	—	16.5	806
2-Ethoxyethanol							
0	10/10	142	333	191		21.2	
1250	10/10	142	331	189	99	20.7	109
2500	10/10	146	325	179	98	19.4	205
5000	10/10	144	315	171	95	18.3	400
10,000	10/10	142	268	127	80	16.6	792
20,000	5/10[5]	143	204	61	61	18.4	2240
2-Butoxyethanol							
0	10/10	137	297	160		22.3	
750	10/10	139	306	167	103	20.9	69
1500	10/10	135	308	173	104	19.6	129
3000	10/10	138	295	157	99	20.5	281
4500	10/10	137	277	140	93	17.7	367
6000	10/10	138	260	122	88	16.4	452

TABLE 4 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (ppm)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	10/10	114	194	79		15.6	
750	10/10	116	194	78	100	14.9	70
1500	10/10	114	174	60	90	13.5	135
3000	10/10	114	148	34	76	13.4	297
4500	5/10[6]	115	153	37	79	16.3	546
6000	0/10[7]	115	–	–	–	13.2	785
2-Ethoxyethanol							
0	10/10	123	197	74		17.9	
1250	10/10	123	194	71	98	16.3	122
2500	10/10	124	190	66	96	16.2	247
5000	10/10	127	186	59	94	14.8	466
10,000	10/10	126	171	45	89	12.4	804
20,000	3/10[8]	126	185	59	94	14.6	2061
2-Butoxyethanol							
0	10/10	110	187	77		18.8	
750	10/10	110	188	78	101	17.1	82
1500	10/10	109	185	76	99	15.5	151
3000	10/10	107	180	73	96	15.2	304
4500	10/10	112	164	52	88	11.8	363
6000	10/10	103	150	47	80	10.7	470

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) × 100.
[4] All males were dead by Week 5 of dosing.
[5] Week of death: 8, 8, 9, 9, 9.
[6] Week of death: unavailable.
[7] All females were dead by Week 7 of dosing.
[8] Week of death: 5, 5, 6, 6, 7, 8, 9.

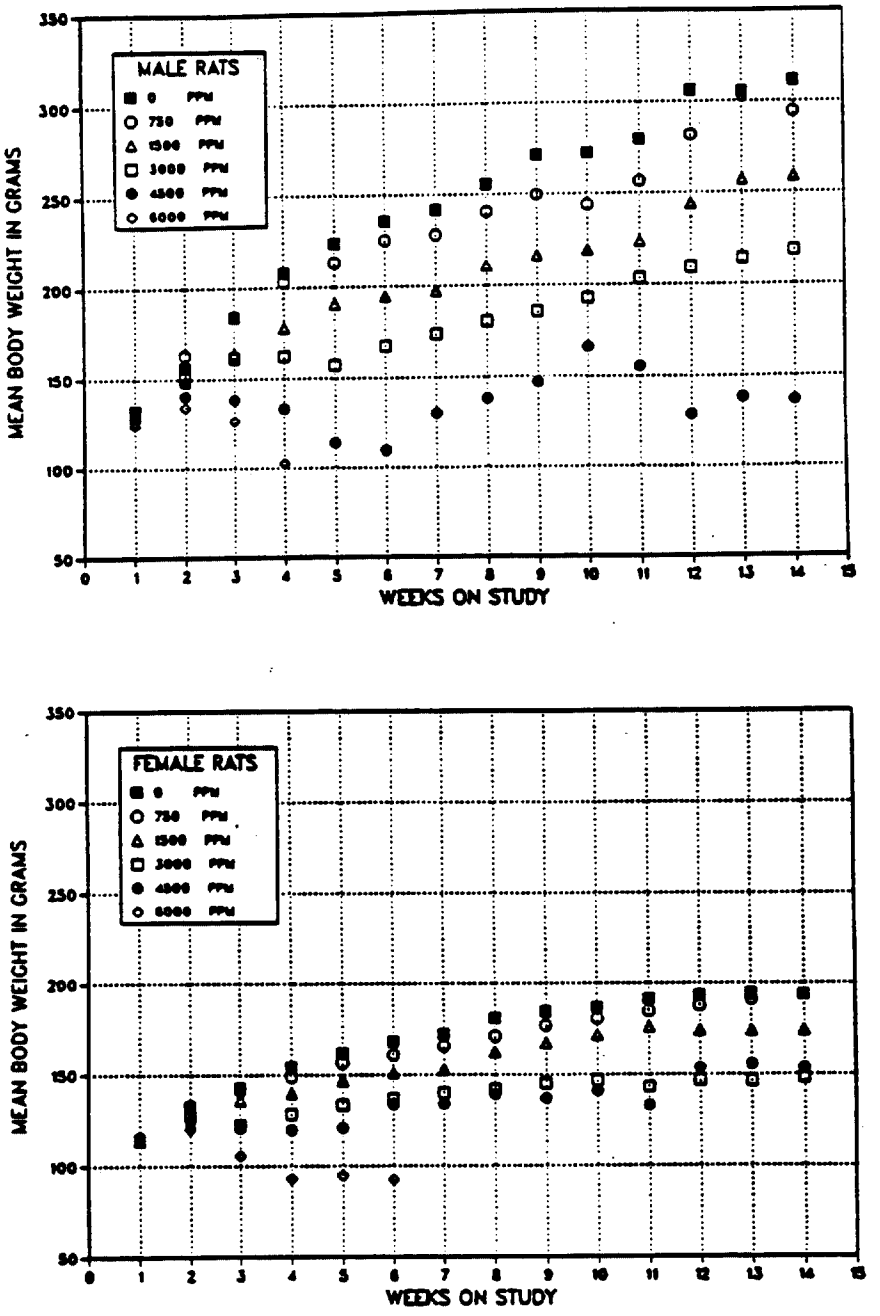

FIGURE 1 **Body Weights of F344/N Rats Administered 2-Methoxyethanol in Drinking Water for 13 Weeks**

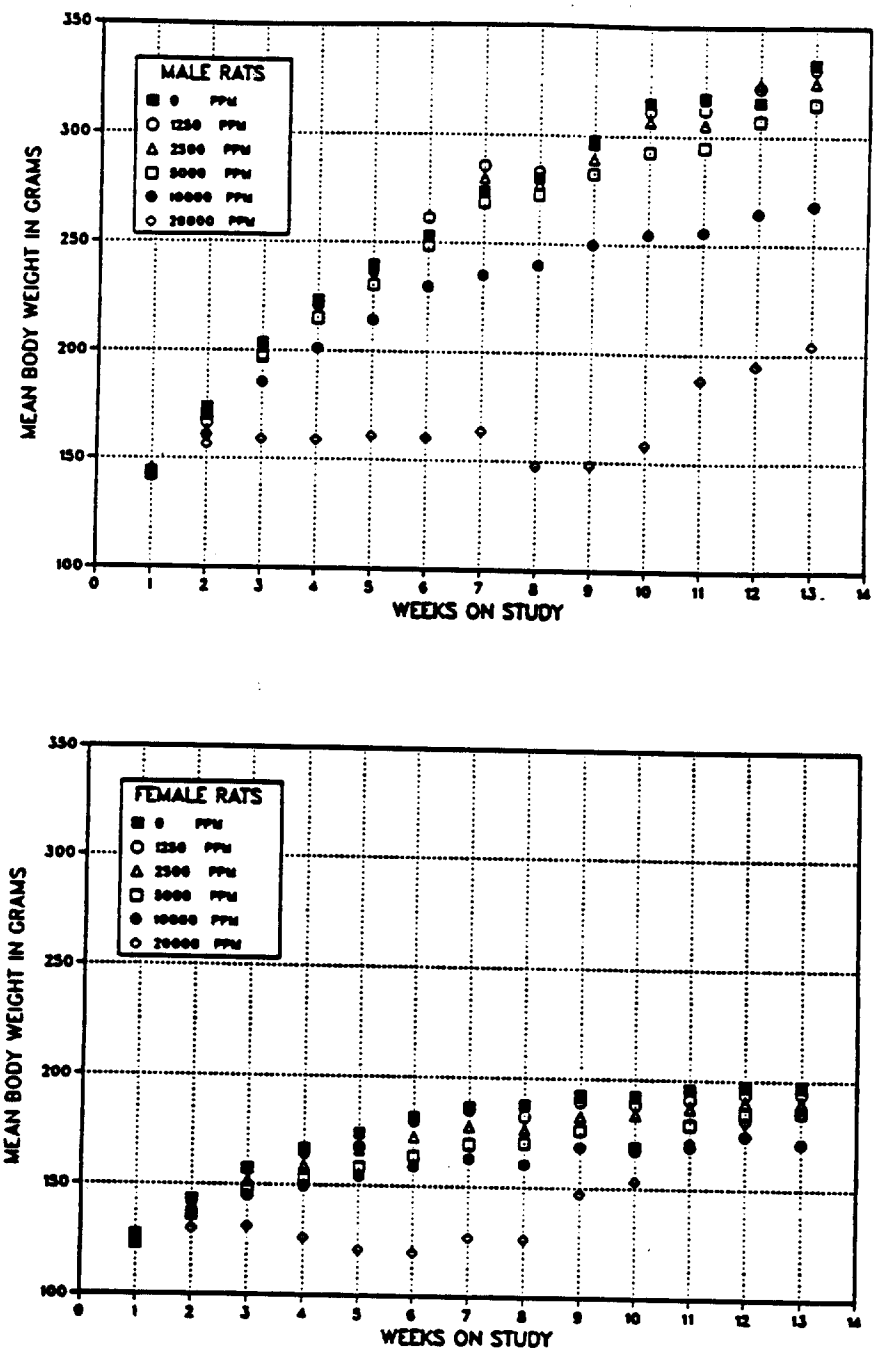

FIGURE 2 Body Weights of F344/N Rats Administered 2-Ethoxyethanol in Drinking Water for 13 Weeks

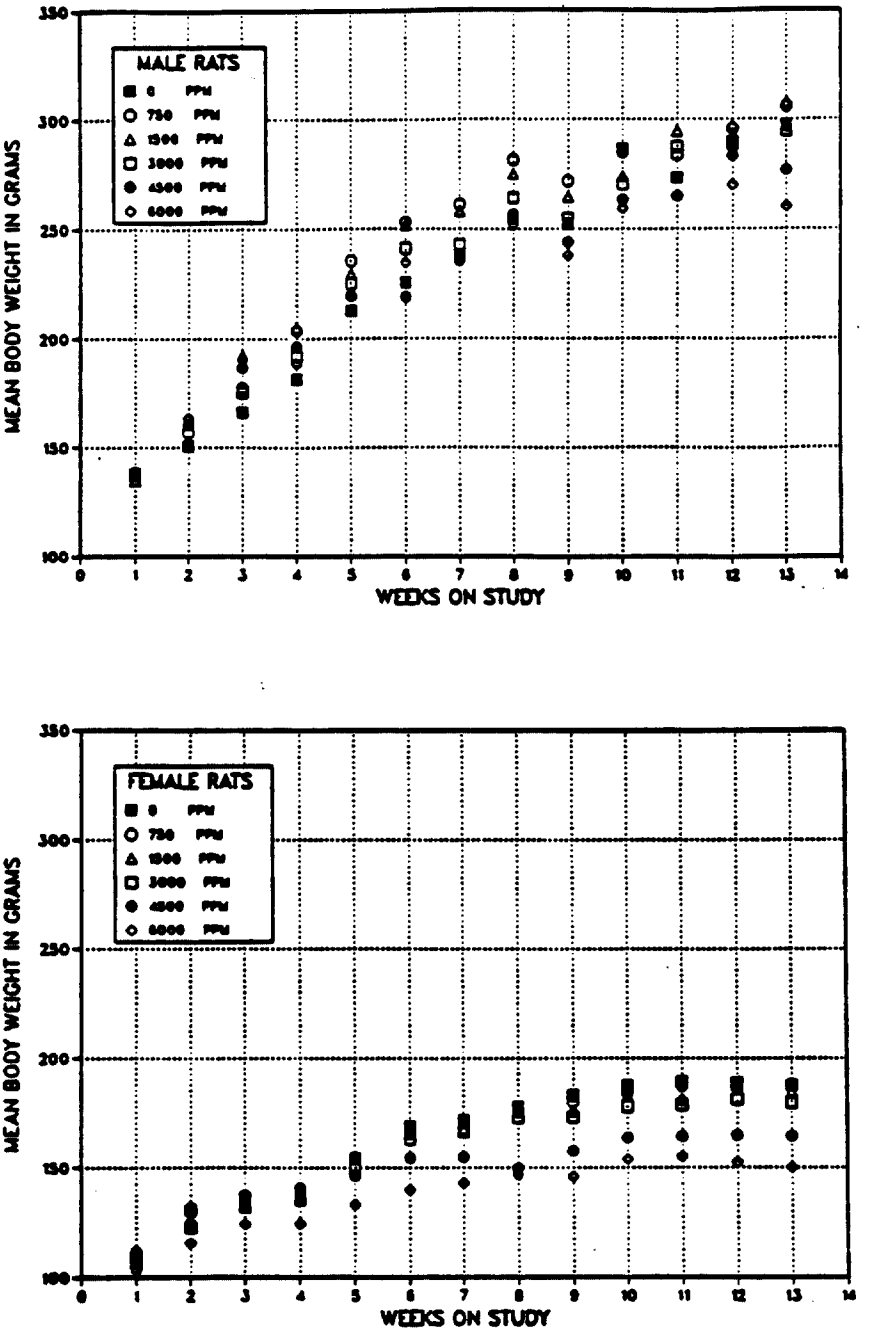

FIGURE 3 Body Weights of F344/N Rats Administered 2-Butoxyethanol in Drinking Water for 13 Weeks

For male and female rats dosed with 2-methoxyethanol, clinical signs of toxicity considered to be chemical-related included tremors, diarrhea, emaciation, abnormal posture, pallor, tachypnea, hypoactivity, and comatose state. Clinical signs noted for male and female rats treated with 2-ethoxyethanol were emaciation, diarrhea, abnormal posture, polyuria, and tremors. For male and female rats treated with 2-butoxyethanol, clinical observations included diarrhea and polyuria.

2-Methoxyethanol: At Week 1 in the hematologic evaluations of 2-methoxyethanol, mild anemia, moderate leukopenia, and moderate thrombocytopenia were present in male rats in the higher dose groups. These animals had decreases in hematocrit (HCT) and hemoglobin (HGB) concentrations and in erythrocyte (RBC), platelet, and total leukocyte counts (Appendix D, Table D1). The anemia was normocytic (no change in mean cell volume), normochromic (no change in mean cell hemoglobin concentration), and poorly regenerative (indicated by a decrease in reticulocyte count). Leukopenia was produced by decreases in neutrophils and lymphocytes. There were moderate decreases in bone marrow cellularity counts in rats in the higher dose groups. At Weeks 3 and 13, the anemia was moderate, progressive, normocytic, and normochromic, with inadequate regeneration (no increase in reticulocyte count). Moderate leukopenia (lymphopenia and neutropenia) and thrombocytopenia were present at each time point, and bone marrow cellularity counts were decreased in male rats in the higher dose groups at Week 13.

Changes in clinical chemistry variables at the various time points for male rats included decreases in creatinine, total protein, albumin, and alkaline phosphatase (AP) (all consistent with decreased food intake) and mild increases in concentrations of bile acids at Weeks 1 and 3.

At Week 1, female rats had a mild normocytic, normochromic, poorly regenerative anemia. At Weeks 3 and 13, the anemia remained mild but, unlike that in male rats, was slightly microcytic (Weeks 3 and 13). Reticulocyte counts were unchanged in the presence of anemia at Weeks 3 and 13 (Appendix D, Table D1). Moderate thrombocytopenia and leukopenia (lymphopenia and neutropenia) occurred at all time points in numerous dose groups. Bone marrow cellularity was decreased by treatment at Weeks 1 and 3 but was unchanged at Week 13.

Clinical chemistry effects in female rats included decreases in AP activity and total protein and albumin concentrations in numerous dose groups at all time points. These findings are consistent with the decreased feed consumption of these animals. Additionally, there were mild increases in concentrations of bile acids in animals in multiple dose groups at Weeks 1 and 3.

For males and females, treatment-related changes in urinalysis parameters consisted of decreases in urine volume and increases in specific gravity.

With the exception of changes in thymus and testis weights, changes in absolute and relative organ weights in the 13-week study of 2-methoxyethanol could probably be attributed to low final mean body weights. Dose-related decreases were noted for the absolute and relative testis weights of male rats and the absolute and relative thymus weights of male and female rats (Table 5). Complete organ weight data for rats treated with 2-methoxyethanol for 13 weeks are presented in Appendix C, Tables C1 and C2.

Almost all observed gross lesions in the 13-week study of 2-methoxyethanol were considered to be secondary to the marked reduction in body weight gain and the overall smaller size of rats administered the higher exposure concentrations of 2-methoxyethanol. The only gross lesion attributed directly to the toxicity of 2-methoxyethanol was a reduction in testis size in males administered 2-methoxyethanol at concentrations of 1500 ppm and greater.

TABLE 5 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	2	0
Necropsy body wt	316	295	260**	214**	136**	–
Right testis						
Absolute	1.398	1.411	0.603**	0.442**	0.254**	–
Relative	4.44	4.81	2.31**	2.07**	1.89*	–
Thymus						
Absolute	0.268	0.198*	0.160**	0.095**	0.072**	–
Relative	0.85	0.67	0.61	0.45**	0.53	–
FEMALE						
n	10	10	10	10	5	0
Necropsy body wt	189	189	170**	145**	151**	–
Thymus						
Absolute	0.224	0.180*	0.125**	0.084**	0.099**	–
Relative	1.19	0.95**	0.74**	0.57**	0.66**	–

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

Histopathologic changes in the testes consisted of a minimal to marked degeneration of germinal epithelium in the seminiferous tubules; in more severely affected rats, the atrophic seminiferous tubules contained only Sertoli cells and a few spermatogonia. The presence of cell debris and a decrease in sperm within the lumen of the epididymis were associated with these changes. Degeneration was present at all dose levels but was only minimal in 7/10 rats in the 750 ppm group (Table 6).

TABLE 6 Incidence and Severity of Selected Histopathologic Lesions In F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	10	10
Bone marrow						
Cellular depletion	0	0	0	0	8 (2.6)	10 (3.0)
Spleen						
Atrophy	0	0	0	0	7[2] (2.4)	10 (2.8)
Capsular fibrosis	0	1 (1.0)	4 (1.5)	10 (2.2)	5[2] (1.2)	1 (1.0)
Thymus						
Atrophy	0	0	3[2] (2.0)	2 (1.5)	9[2] (3.1)	9[2] (3.6)
Testes						
Degeneration	0	7 (1.0)	10 (2.6)	10 (4.0)	9 (4.0)	10 (4.0)
Prostate						
Atrophy	0	0	0	0	9 (2.2)	10 (2.7)
Preputial gland						
Atrophy	0	0	0	1 (1.0)	9 (2.1)	8[3] (2.8)
Bone, metaphysis						
Atrophy	0	–[4]	0[5]	0	9[2] (3.0)	10 (3.0)
FEMALE						
n	10	10	10	10	10	10
Bone marrow						
Cellular depletion	0	0	1 (1.0)	7 (1.6)	6 (1.8)	9 (3.6)
Spleen						
Atrophy	0	0	1 (2.0)	1 (1.0)	5 (1.8)	10 (2.3)
Capsular fibrosis	0	0	3 (1.0)	5 (1.2)	0	0
Thymus						
Atrophy	0	0	1 (1.0)	9 (1.4)	7[2] (2.3)	10 (3.6)
Uterus						
Atrophy	0	0	0	8 (2.6)	9 (2.7)	10 (2.9)
Ovary						
Atrophy	0	0	0	6 (1.5)	10 (2.3)	10 (3.1)
Clitoral gland						
Atrophy	0	0	0	4[3] (1.8)	8 (2.6)	8[3] (2.8)
Bone, metaphysis						
Atrophy	0	–	–	0	10 (3.0)	10 (3.0)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

[2] n=9.

[3] n=8.

[4] Not applicable; tissue not examined for animals in this dose group.

[5] n=2.

Additionally, a chemical-related fibrosis of the splenic capsule was present in male and female rats (Plates 1 and 2) and was most prominent in animals in the 1500 to 4500 ppm groups. This fibrosis was characterized by focal areas in which there was thickening of the splenic capsule by fibrous connective tissue and a minimal mixed inflammatory cell infiltrate; inflammation and fibrosis of the serosal surfaces of other abdominal organs did not occur.

Other microscopic changes were associated with the marked reduction in body weight gain or stress-related physiological changes typically seen in animals that die during study or are killed moribund. Specifically, these changes included atrophy of the clitoral/preputial glands, uterus, ovary, salivary glands, and prostate (Table 6). Atrophic changes included not only an overall reduction in the size of the organs but a depletion of secretory product in the lumen of glands, decreased height of the secretory epithelium, and an increased number of degenerative and apoptotic cells. Lymphoid depletion (atrophy) in lymph nodes, thymus, and spleen, bone marrow depletion, absence of metaphyseal bone growth, focal erosion/ulcerations of the glandular stomach, and focal proliferation of bacterial or fungal organisms were also seen in animals that died or were euthanized during the study; these lesions were considered to be secondary to the marked generalized toxicity and reduction in body weight gain seen in the 4500 and 6000 ppm 2-methoxyethanol groups.

A summary of lesions in rats in the 13-week drinking water study of 2-methoxyethanol is presented in Appendix A, Tables A1 and A2.

In the 13-week study of 2-methoxyethanol, sperm morphology evaluations were performed on male rats treated with 0, 750, 1500, or 3000 ppm, and vaginal cytology evaluations were performed on female rats treated with 0, 1500, 3000, or 4500 ppm. Testicular and epididymal weights were significantly lower than control values for males receiving 1500 or 3000 ppm 2-methoxyethanol (Appendix E, Table E1). Also, spermatozoal measurements were significantly decreased for males in the 2 highest dose groups (1500 or 3000 ppm). There were no significant differences from control in estrous cycle length for females treated with 2-methoxyethanol (Appendix E, Table E2). However, there was evidence to suggest that animals in the 1500 and 3000 ppm groups differed from the control animals in the relative frequency of time spent in estrous stages. The lack of significance at the

4500 ppm dose level may have been due to increased variability and/or the small sample size (5 females).

2-Ethoxyethanol: At Week 1 in the hematologic evaluations of 2-ethoxyethanol, male rats exhibited a mild anemia (indicated by decreases in RBC count and HGB concentration) that was macrocytic (increase in mean cell volume), hypochromic (decrease in mean cell hemoglobin concentration), and poorly regenerative. Hypochromia resulted from an increase in cell size (swelling), which kept the HCT high relative to the HGB concentration, and not from an increase in numbers of large, young RBCs (reticulocytes), which, in fact, were markedly reduced (and, typically, are normochromic) (Appendix D, Table D2). Mild thrombocytopenia and leukopenia (produced by moderate lymphopenia and mild neutrophilia) were present, and a moderate decrease in bone marrow cellularity occurred in males in the 10,000 ppm group. There were mild decreases in total protein and albumin concentrations, as well as a moderate decrease in AP activity.

At Weeks 3 and 13, the anemia in male rats was moderate to marked (indicated by decreases in HCT and HGB concentrations and RBC count), macrocytic, normochromic, and regenerative. Mild thrombocytopenia was present at Week 3 but absent at Week 13. Moderate leukopenia (produced by lymphopenia and neutropenia) persisted at Week 3, but marked leukocytosis (lymphocytosis and neutrophilia) appeared to be present at Week 13. Bone marrow cellularity was unchanged at Week 3 and increased in males in the 10,000 ppm group at Week 13. Clinical chemistry findings at these time points consisted of mild decreases in total protein and albumin concentrations and moderate decreases in AP activity. Concentrations of total bile acids increased significantly in males in the 2 highest dose groups (10,000 and 20,000 ppm) at Week 3 but were unchanged at Week 13.

As in male rats, a mild anemia (indicated by decreases in RBC count and HGB concentration) was noted in female rats at Week 1 that was macrocytic (increase in mean cell volume), hypochromic (decrease in mean cell hemoglobin concentration), and poorly regenerative (decrease in reticulocyte count) (Appendix D, Table D2). These rats had a moderate to marked thrombocytopenia and moderate leukopenia (lymphopenia). Bone

marrow cellularity counts were not affected. Clinical chemistry findings consisted of mild decreases in total protein and albumin concentrations and in AP activity.

At Weeks 3 and 13, the anemia progressed from mild to moderate and remained macrocytic (marked at 13 weeks), regenerative (marked at 13 weeks), and mildly hypochromic. Thrombocytopenia was moderate at each time point, and the moderate leukopenia (lymphopenia and neutropenia) at Week 3 appeared to be replaced by marked leukocytosis (neutrophilia and lymphocytosis) at Week 13. Bone marrow cellularity counts did not change at Week 3 but were significantly increased in animals in multiple dose groups at Week 13. Decreases in total protein concentration and AP activity were similar to those noted in male rats. At Week 3, alanine aminotransferase activity and concentrations of total bile acids were significantly increased in females in the 3 highest dose groups (5000, 10,000, and 20,000 ppm), and creatinine kinase activity was significantly increased in females in the 4 highest dose groups (2500, 5000, 10,000, and 20,000 ppm). Mild hepatocellular alterations were present at Week 3, but these effects were not detected at Week 13.

For rats treated with 2-ethoxyethanol, treatment-related changes in urinalysis parameters, when present, involved decreases in urine volume and increases in specific gravity.

In the 13-week study of 2-ethoxyethanol, no organ weight analyses were performed for male or female rats in the 20,000 ppm groups due to the high mortality at this exposure level. For the remaining dose groups, changes in absolute and relative organ weights could probably be attributed to low final mean body weights, excluding decreases noted in absolute and relative thymus and testis weights. Absolute and relative thymus weights decreased in a dose-related fashion for males and females, and absolute and relative testis weights for males in the 10,000 ppm 2-ethoxyethanol group were significantly lower than those of the control group (Table 7). Complete organ weight data for rats treated with 2-ethoxyethanol for 13 weeks are presented in Appendix C, Tables C1 and C2.

TABLE 7 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	1250	2500	5000	10,000	20,000
MALE						
n	10	10	10	10	10	0
Necropsy body wt	315	309	296**	295*	236**	–
Right testis						
Absolute	1.394	1.431	1.443	1.342	0.618**	–
Relative	4.43	4.64	4.89	4.56	2.62*	–
Thymus						
Absolute	0.299	0.270	0.213**	0.256**	0.154**	–
Relative	0.95	0.87	0.72**	0.87*	0.65**	–
FEMALE						
n	10	10	10	10	10	0
Necropsy body wt	185	183	177	173**	149**	–
Thymus						
Absolute	0.214	0.210	0.221	0.186	0.069**	–
Relative	1.16	1.15	1.25	1.07	0.47**	–

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

In the 13-week study of 2-ethoxyethanol, the only chemical-related gross lesion noted in rats was a reduction in testis size in males in the 10,000 and 20,000 ppm groups. Microscopic changes in the testis were morphologically similar to those seen in the 2-methoxyethanol study in rats and consisted of a minimal to marked degeneration of germinal epithelium in the seminiferous tubules. In more severely affected animals, the atrophic tubules contained only Sertoli cells and a few spermatogonia. At the highest dose (20,000 ppm), there was a decrease in the size of the interstitial cells compared to those of the control group. Testicular degeneration was present in all male rats administered 2-ethoxyethanol at concentrations of 5000 ppm or greater for 13 weeks (Table 8). At the 5000 ppm exposure level, the severity of degeneration was minimal; although degeneration was present in a few tubules throughout the testes, there was no apparent histopathologic

effect on the majority of seminiferous tubules. At the 2 highest exposure levels (10,000 and 20,000 ppm), the severity of degeneration was moderate to marked.

Chemical-related lesions at other sites that were related to hematologic toxicity included increased hematopoiesis and hemosiderin pigmentation in the spleen (Plate 3), increased bone marrow hematopoiesis, and increased hemosiderin pigmentation in Kupffer's cells of the liver (Table 8). Other microscopic changes in rats were associated with the marked reduction in body weight gain or physiological stress-related changes typically seen in animals that die or are euthanized because of moribund condition. These changes, present almost exclusively at the highest dose, included atrophy of the clitoral/preputial glands, uterus, ovary, salivary glands, seminal vesicle and, prostate. Lymphoid depletion in the lymph nodes, thymus, and spleen was also noted.

A summary of lesions in rats in the 13-week drinking water study of 2-ethoxyethanol is presented in Appendix A, Tables A3 and A4.

TABLE 8 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	1250	2500	5000	10,000	20,000
MALE						
n	10	10	10	10	10	5
Liver						
Degeneration	0	0	0	0	0	5 (2.4)
Pigmentation	0	0	0	0	10 (1.0)	5 (1.0)
Hematopoiesis	0	0	0	0	9 (1.7)	0
Bone marrow						
Cellular depletion	0	0	0	0	0	5 (3.6)
Hyperplasia	0	0	0	0	10 (2.7)	0
Spleen						
Hematopoiesis	0	0	0	10 (2.0)	10 (3.2)	0
Pigmentation	0	0	0	0	0	5 (2.6)
Atrophy	0	0	0	0	0	4 (2.3)
Thymus						
Atrophy	0	0[2]	0[3]	0	4 (2.0)	2[4] (4.0)
Testes						
Degeneration	0	0	0	10 (1.1)	10 (3.5)	5 (4.0)
Prostate						
Atrophy	0	0	6 (1.3)	7 (1.4)	10 (2.0)	5 (3.4)
FEMALE						
n	10	10	10	10	10	7
Liver						
Degeneration	0	0	0	0	0	6 (1.8)
Pigmentation	0	0	0	0	10 (1.0)	7 (1.0)
Hematopoiesis	0	0	0	0	9 (2.0)	0
Bone marrow						
Cellular depletion	0	0	0	0	0	7 (3.3)
Hyperplasia	0	0	0	0	10 (3.0)	0
Spleen						
Hematopoiesis	0	0	0	0	10 (2.5)	0
Pigmentation	0	0	0	0	0	7 (2.7)
Atrophy	0	0	0	0	0	6 (2.2)
Thymus						
Atrophy	0	–[5]	–	0	10 (1.3)	6[6] (4.0)
Uterus						
Atrophy	0	0	0	0	9 (2.7)	7 (3.7)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] n=1.
[3] n=2.
[4] n=3.
[5] Not applicable; tissue not examined for animals in this dose group.
[6] n=6.

Sperm morphology and vaginal cytology evaluations were performed on rats receiving 0, 2500, 5000, or 10,000 ppm 2-ethoxyethanol. Testicular weights were significantly lower than the control value for males in the highest dose group (10,000 ppm), and epididymal weights were significantly lower than those of the control group for males receiving 5000 or 10,000 ppm 2-ethoxyethanol (Appendix E, Table E3). All spermatozoal measurements were significantly less than those of the control group for males in the 10,000 ppm group, and sperm concentration was also significantly less than that of the control group for males treated with 2500 or 5000 ppm 2-ethoxyethanol. There was a significant decrease in estrous cycle length compared to the control value for females receiving 10,000 ppm 2-ethoxyethanol (Appendix E, Table E4). Evidence suggested that animals in this dose group differed significantly from the controls in the relative frequency of time spent in estrous stages, with females in the 10,000 ppm group spending more time in diestrus and less time in proestrus and estrus than did control animals.

2-Butoxyethanol: At all time points in the hematologic evaluations of 2-butoxyethanol, mild anemia (indicated by a decrease in RBC counts) was present in male rats in the 3 highest dose groups (3000, 4500, and 6000 ppm), and thrombocytopenia was present in males in the 2 highest dose groups (4500 and 6000 ppm). Decreases in HGB concentration were mild at Weeks 1 and 13 and sporadic at Week 3 (Appendix D, Table D3). There were no consistent changes in HCT. The anemia was markedly macrocytic and mildly hypochromic at each time point, and reticulocyte counts were moderately increased at Weeks 1 and 13. Leukocyte counts appeared to be mildly to markedly increased (lymphocytosis and neutrophilia) at Week 1 in male rats in the 3 highest dose groups and unchanged at successive time points. Bone marrow cellularity was mildly increased in the 2 highest dose groups at Week 1. Clinical chemistry effects included mild increases in total protein and albumin in males in multiple dose groups at Week 1 and decreases of similar magnitude at Week 13. AP activity increased in male rats in multiple groups at Week 1 and in the highest dose group (6000 ppm) at Week 3. Increased AP activity is consistent with mild cholestasis.

In female rats, there was mild to moderate anemia (indicated by decreases in RBC counts and, less consistently, HCT and HGB concentrations) in most dose groups at each time point (Appendix D, Table D3). The anemia was markedly macrocytic, mildly to moderately

hypochromic (normochromic at Week 1), and regenerative (with the exception of Week 3 reticulocyte counts, which were not increased). Platelet counts were mildly increased in animals in the higher dose groups at Week 1 but were decreased at Weeks 3 and 13. Marked leukocytosis (neutrophilia and lymphocytosis) appeared to be present at Week 1. There were mild increases in bone marrow cellularity in female rats in the higher dose groups at Weeks 1 and 13. Changes in clinical chemistry variables included moderate, consistent increases in concentrations of urea nitrogen and creatinine (mild, less prevalent) at Weeks 3 and 13 and mild decreases in concentrations of total protein and albumin at these same time points. AP activity was mildly increased in rats in the high-dose group at Week 1 and in the 2 highest dose groups at Week 13.

For male and female rats treated with 2-butoxyethanol, treatment-related changes in urinalysis parameters consisted of decreases in urine volume and increases in specific gravity.

In the 13-week study of 2-butoxyethanol in rats, the absolute thymus weights of males in the 4500 ppm group and males and females in 6000 ppm groups were significantly lower than those of the control groups. Other changes noted in absolute and relative organ weights were believed to be secondary to changes in body weight. Complete organ weight data for rats treated with 2-butoxyethanol for 13-weeks are presented in Appendix C, Tables C1 and C2.

In the 2-butoxyethanol study, the only gross lesion considered to be chemical-related was a reduction in the size of the uterus of female rats in the 4500 and 6000 ppm groups. Microscopically, there was minimal to mild uterine atrophy characterized by a decreased thickness of the muscular wall and uterine mucosa. This was considered to be secondary to the reduction in body weight gain rather than a direct chemical effect of 2-butoxyethanol.

Chemical-related histopathologic lesions occurred in the liver, spleen, and bone marrow of male and female rats. Liver lesions included cytoplasmic alteration, hepatocellular degeneration, and pigmentation. All of these lesions were present in the majority of dosed rats, but they were more prominent in the 3 highest dose groups (3000, 4500, and

6000 ppm); lesions were slightly more severe in females (Table 9). Cytoplasmic alteration in the liver of 2-butoxyethanol-dosed rats was characterized by hepatocytes that stained more eosinophilic and lacked the amphophilic to basophilic granularity of the cytoplasm typically present in controls. Hepatocellular degeneration was primarily centrilobular and was characterized by a variety of changes, including the occasional intensely eosinophilic-stained hepatocyte and hepatocytes that appeared shrunken with angular cytoplasmic borders and a densely stained nucleus (Plate 4). Pigmentation was present in Kupffer's cell cytoplasm, primarily in the centrilobular region. This brown to green granular pigment stained strongly positive for iron; some of the pigment granules also stained weakly positive by the PAS method. Hyperplasia of the bone marrow in dosed rats consisted of increased cellularity of hematopoietic cells in the mid shaft of the femur with a decrease in the amount of marrow fat cells relative to that seen in controls. A corresponding increase in hematopoiesis and hemosiderin pigment was also present in the spleen.

A summary of lesions in rats in the 13-week drinking water study of 2-butoxyethanol is presented in Appendix A, Tables A5 and A6.

Male and female rats treated with 0, 3000, 4500, or 6000 ppm 2-butoxyethanol were evaluated for sperm morphology and vaginal cytology. Results showed a decrease in left epididymal weight for males in the 4500 and 6000 ppm groups (Appendix E, Table E5). The only spermatozoal measurement that showed a significant change relative to the control group was sperm concentration, which was decreased in all treated males. There were no significant differences from the control group in estrous cycle length for treated females (Appendix E, Table E6). However, evidence suggested that animals in the 4500 and 6000 ppm groups differed significantly from the controls in the amount of time spent in estrous stages, with females in these 2 groups spending more time in diestrus and less time in proestrus, metestrus, and estrus than did control animals.

TABLE 9 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	4 (1.0)	8 (1.0)	7 (1.1)	10 (2.0)	10 (1.8)
Degeneration	0	0	0	8 (1.0)	8 (1.0)	10 (1.0)
Pigmentation	0	0	0	0	0	7 (1.0)
Bone marrow						
Hyperplasia	0	0	0	2 (1.0)	2 (2.0)	8 (2.0)
Spleen						
Hematopoiesis	0	0	0	0	2 (1.0)	2 (1.0)
Pigmentation	0	0	2 (1.0)	10 (1.1)	8 (1.4)	10 (2.0)
FEMALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	5 (1.4)	9 (2.0)	10 (2.2)	10 (3.0)	10 (3.0)
Degeneration	0	0	0	10 (1.3)	10 (1.3)	10 (1.1)
Pigmentation	0	0	2 (1.0)	10 (1.2)	10 (1.9)	10 (1.9)
Bone marrow						
Hyperplasia	0	0	0	0	4 (2.0)	3 (2.0)
Spleen						
Hematopoiesis	0	0	0	0	6 (1.2)	10 (1.0)
Pigmentation	0	0	1 (2.0)	9 (2.0)	10 (2.0)	9 (2.0)
Uterus						
Atrophy	0	0	0	1 (1.0)	9 (1.2)	8 (2.0)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

Stop-Exposure Studies in Male F344/N Rats

All rats treated with 6000 ppm 2-methoxyethanol died by Week 6 of the study. For rats treated with 2-ethoxyethanol, 20 of 30 animals in the 20,000 ppm group died or were killed before the scheduled 60-day evaluation. One death in each of the 10,000 and 20,000 ppm groups was recorded after treatment with 2-ethoxyethanol was discontinued (Table 10). Due to the excessive mortality in males receiving 20,000 ppm 2-ethoxyethanol in both the stop-exposure and 13-week base studies, the 5 surviving rats in the 20,000 ppm base-study group were combined with the 10 surviving rats in the 20,000 ppm stop-exposure group at Day 60 of the stop-exposure study. No rats treated with 2-butoxyethanol died or were killed prior to the scheduled terminations.

Due to 100% mortality in the 6000 ppm 2-methoxyethanol group, mean body weights and weight changes were not determined for rats in this dose group after Week 6 of the study. However, at the Day 60 evaluation, mean body weights for rats in the 1500 and 3000 ppm 2-methoxyethanol groups were notably lower than those of the control group (Table 10). Although rats in these dose groups gained more weight than controls from Day 60 to the end of the recovery period, final mean body weights for rats in the 1500 and 3000 ppm 2-methoxyethanol groups remained at least 9% less than the control value (Figure 4).

In the 2-ethoxyethanol stop-exposure study, Day 60 mean body weights were at least 6% lower than the control value for rats in all treated groups, and the Day 60 mean body weight of rats in the 20,000 ppm group was 48% lower than the control value (Table 10). Mean body weight changes at Day 60 were also markedly lower in rats treated with 10,000 or 20,000 ppm 2-ethoxyethanol. During the recovery period, rats in the 10,000 and 20,000 ppm groups gained more weight than controls. However, final mean body weights in all treated groups were still at least 7% lower than that of the control group; the final mean body weight of rats in the 20,000 ppm group was 29% lower than the control value (Figure 5).

TABLE 10 Survival, Weight Gain, Water Consumption, and Compound Consumption in Male F344/N Rats in the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams)				Weight Relative to Controls (%)[3]		Water Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
		Initial	Day 60	Final	Change[2]	Day 60	Final		
2-Methoxyethanol									
0	10/30	142	303	379	237			20.7	
1500	10/30	136	253	346	210	83	91	20.3	123
3000	10/30	144	223	329	185	74	87	17.5	255
6000	0/30[6]	143	—	—	—	—	—	16.5	745
2-Ethoxyethanol									
0	10/30	164	302	388	224			21.2	
5000	10/30	164	284	361	197	94	93	19.3	407
10,000	9/30[7]	165	256	353	188	84	91	17.5	792
20,000	5/35[8]	161	157	277	116	52	71	19.9	2390
2-Butoxyethanol									
0	10/30	147	289	356	209			21.1	
1500	10/30	144	295	363	219	102	102	20.2	124
3000	10/30	150	284	342	192	98	96	19.8	234
6000	10/30	147	261	329	182	90	92	19.7	443

[1] Number surviving at Day 116/number of rats per group. Number surviving does not include animals killed at the Day 60 and 90 evaluations.
[2] Mean weight change from study start to study end.
[3] (Dose group mean/control group mean) x 100.
[4] Average water consumed per dose group from study start to study end.
[5] Average compound consumption during Days 0 to 60 of study.
[6] All rats in this group died before Day 60.
[7] One rat in this group died after Day 60.
[8] Twenty rats in this group died at or before Day 60; 1 rat died after Day 60. Because of the excessive mortality in rats administered 20,000 ppm 2-ethoxyethanol in both the stop-exposure and 13-week base studies, the 5 surviving base study rats were moved to the 20,000 ppm stop-exposure group at Day 60.

In the 2-butoxyethanol stop-exposure study, the mean body weight of rats in the 6000 ppm group at Day 60 and at the study end was notably lower than that of the control group; however, the mean body weight of rats in this dose group remained within 10% of the control value at both time points (Table 10). The mean body weights of rats in the 1500 and 3000 ppm groups were similar to the control value at Day 60 and study end (Figure 6).

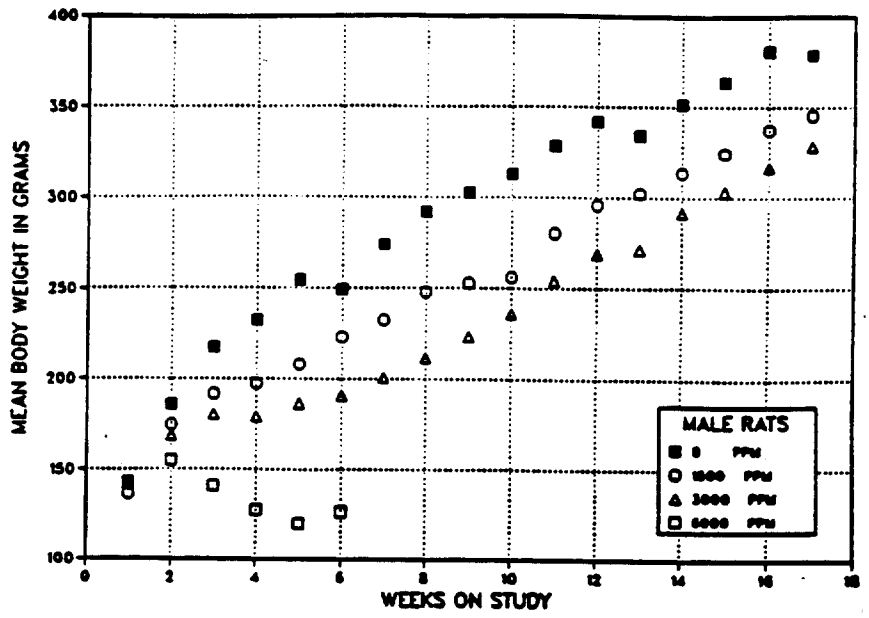

FIGURE 4 **Body Weights of Male F344/N Rats Administered 2-Methoxyethanol in Drinking Water for 60 Days**

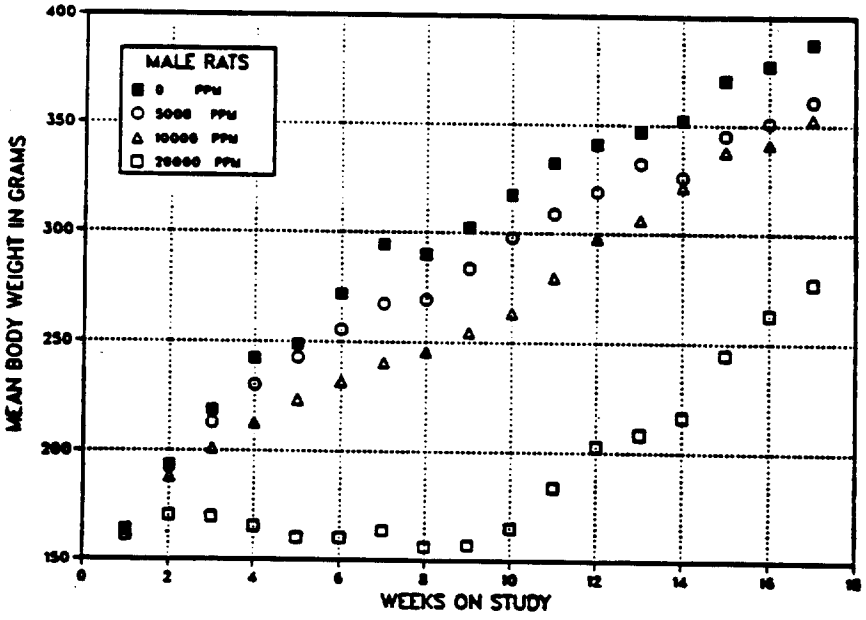

FIGURE 5 **Body Weights of Male F344/N Rats Administered 2-Ethoxyethanol in Drinking Water for 60 Days**

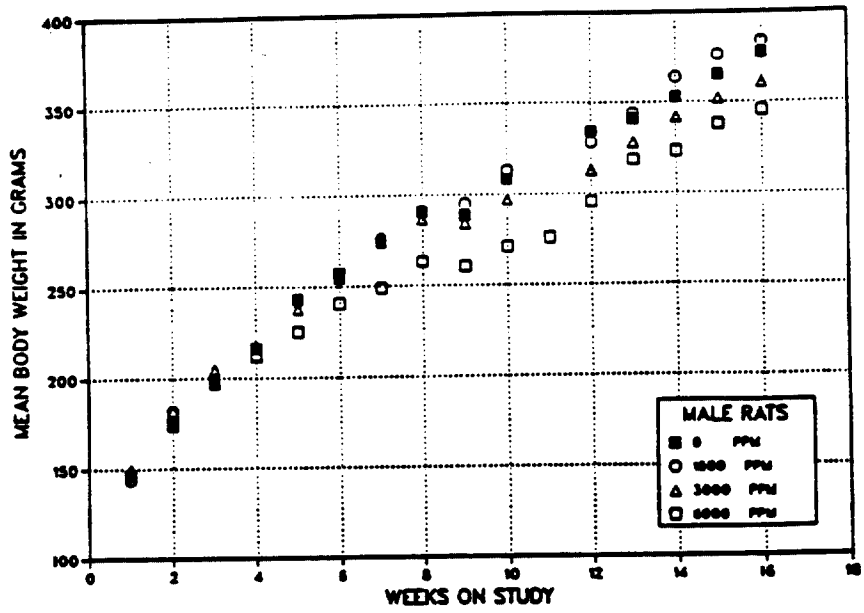

FIGURE 6 Body Weights of Male F344/N Rats Administered 2-Butoxyethanol in Drinking Water for 60 Days

For male rats treated with 2-methoxyethanol or 2-butoxyethanol, a dose-related decrease was noted in mean daily water consumption. Mean daily water consumption was also decreased for rats in all 2-ethoxyethanol dose groups. Average compound consumption increased with dose for rats treated with the ethylene glycol ethers for 60 days (Table 10). Over the course of the 60-day exposure period, compound consumption decreased slightly in rats in the 1500 and 3000 ppm 2-methoxyethanol groups, the 5000 and 10,000 ppm 2-ethoxyethanol groups, and in all 2-butoxyethanol-treated groups.

Clinical observations for rats treated with 2-methoxyethanol were abnormal posture, emaciation, and tachypnea. Clinical observations noted for animals treated with 2-ethoxyethanol included abnormal posture, diarrhea, emaciation, and polyuria. Clinical observations noted for animals treated with 2-butoxyethanol were sporadic and did not appear to be treatment related.

2-Methoxyethanol: For male rats treated with 1500 or 3000 ppm 2-methoxyethanol, absolute and relative testis and epididymal weights were significantly lower than the values for the control group at the 60, 90 and 116 day evaluations (Appendix C, Table C5).

In the stop-exposure study of 2-methoxyethanol, microscopic evaluations were performed on rats after 60 days of exposure to 1500 or 3000 ppm 2-methoxyethanol and after recovery periods of 30 days (Study Day 90) and 56 days (Study Day 116); a 6000 ppm group was initially included in the stop-exposure study, but all rats died prior to the end of the 60-day exposure period. Degeneration of the seminiferous tubules was present in rats in the 1500 and 3000 ppm groups at the end of the 60-day exposure period (Plates 5-8). Degeneration of the seminiferous tubules was also present in rats in the 6000 ppm group that died before the end of the exposure period. The severity of degeneration was marked in all rats from the 3000 and 6000 ppm groups and mild to moderate in rats in the 1500 ppm group (Table 11). In the 2 highest exposure groups, the seminiferous tubules contained only a few spermatogonia and Sertoli cells; there was no evidence of active spermatogenesis in the seminiferous tubules. The lumen of the epididymis contained degenerative cells from the seminiferous tubules and only a few spermatozoa relative to controls. In rats in the 3000 ppm group, there was no evidence of recovery from the testicular degeneration after 30 days of recovery; after 56 days of recovery, all rats had degenerative lesions (mild to marked severity), but some tubules appeared relatively normal, and the lumen contained mature spermatids. In the 1500 ppm group, there was some recovery from the degenerative lesion in the testis after 30 days, but minimal to mild lesions were still present in all rats. After 56 days, there was no evidence of further recovery; all rats had minimal to mild degenerative lesions.

TABLE 11 Incidence and Severity of Testicular Degeneration in Male F344/N Rats in the Stop-Exposure Drinking Water Studies of 2-Methoxyethanol and 2-Ethoxyethanol[1]

	Dose (ppm)			
	0	1500	3000	6000
2-Methoxyethanol				
60 Days	0/10	10/10 (2.4)	10/10 (4.0)	30/30 (4.0)
90 Days	0/10	10/10 (1.2)	10/10 (3.9)	–[2]
116 Days	0/10	10/10 (1.3)	10/10 (3.0)	–
	Dose (ppm)			
	0	5000	10,000	20,000
2-Ethoxyethanol				
60 Days	0/10	0/10	10/10 (2.9)	24/24 (4.0)
90 Days	0/10	6/10 (1.0)	11/11 (2.7)	5/5 (4.0)
116 Days	0/10	7/10 (1.0)	9/9 (2.7)	5/5 (4.0)

[1] Incidences are given as the number of animals with lesions/number of animals examined microscopically. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] Not applicable; all animals died during the 60-day exposure period.

2-Ethoxyethanol: For rats that were treated with 10,000 or 20,000 ppm 2-ethoxyethanol, absolute and relative right testis and epididymal weights were significantly lower than the control values at Days 60, 90, and 116. Also, the absolute testis weight of males treated with 5000 ppm 2-ethoxyethanol was significantly lower than that of the control group at Day 116 (Appendix C, Table C5).

In the stop-exposure study of 2-ethoxyethanol, moderate to marked testicular degeneration was present in rats in the 10,000 and 20,000 ppm groups, but not in the 5000 ppm group, after the 60-day exposure period (Table 11). At the 30 and 56 day recovery periods, there was no evidence of recovery from the testis lesions in these groups. Although no degeneration was evident in the testis of rats from the 5000 ppm group when the exposure was stopped (Day 60), minimal degeneration, similar to that seen at this dose level in the base study, was present in most male rats at the 30 and 56 day recovery periods.

2-Butoxyethanol: In the 2-butoxyethanol stop-exposure study, the only significant organ weight findings were decreases at Day 60 for the absolute testis weights of males in all treated groups and increases in the relative testis and epididymal weights of males in the 6000 ppm group. Organ weights were not evaluated at Day 116 for males treated with 2-butoxyethanol. No chemical-related microscopic lesions were noted in the testis or epididymis of rats in the stop-exposure study of 2-butoxyethanol.

2-Week Studies in B6C3F$_1$ Mice

No male or female mice treated with 2-methoxyethanol or 2-butoxyethanol and no female mice treated with 2-ethoxyethanol died or were killed before the end of the study. One male in the 900 mg/kg 2-ethoxyethanol group died on Day 10 of the 2-week study (Table 12). The final mean body weights for both males and females and the mean body weight gains for females receiving 2-methoxyethanol were not notably different from control values. However, the mean body weight gain for males receiving 1200 mg/kg 2-methoxyethanol was notably lower than the control value. The mean body weights and mean body weight changes for males and females treated with 2-ethoxyethanol or 2-butoxyethanol were variable and did not differ from the controls.

Average water consumption decreased for all males treated with 2-methoxyethanol and for females treated with 200, 400, 1000, or 1200 mg/kg 2-methoxyethanol (Table 12). For female mice treated with 600 mg/kg 2-methoxyethanol, water consumption was increased relative to that of the control group. In the 2-ethoxyethanol study, average water consumption was similar or somewhat increased in males in all treated groups excluding the 2500 mg/kg group; average water consumption for males in this dose group and females in all 2-ethoxyethanol dose groups was decreased. In the 2-butoxyethanol study, decreases in water consumption were noted for males in the 100, 250, and 650 mg/kg groups, and increases in consumption were noted for males in the 150 and 400 mg/kg groups. The average water consumption of females in the 2-butoxyethanol study was decreased at all dose levels excluding the 650 mg/kg level, where consumption was increased. As shown in Table 12, average compound consumption increased with dose in male and female mice treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol.

TABLE 12 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F, Mice in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
2-Methoxyethanol							
0	5/5	24.2	25.3	1.1		5.2	
200	5/5	24.7	26.2	1.5	104	3.9	181
400	5/5	24.3	25.9	1.6	102	4.2	380
600	5/5	24.6	25.7	1.1	102	4.2	603
1000	5/5	25.4	25.5	0.1	101	3.6	865
1200	5/5	24.4	23.6	-0.8	93	3.8	1269
2-Ethoxyethanol							
0	5/5	22.1	24.6	2.5		4.5	
300	5/5	22.0	24.7	2.7	100	5.7	415
600	5/5	21.7	24.0	2.3	98	4.6	850
900	4/5[4]	22.2	25.6	3.4	104	5.8	1140
1500	5/5	22.2	25.0	2.8	102	4.6	1633
2500	5/5	22.6	25.0	2.4	102	4.0	2583
2-Butoxyethanol							
0	5/5	25.4	24.9	-0.5		4.3	
100	5/5	26.0	26.8	0.8	108	4.3	93
150	5/5	26.2	26.4	0.2	106	4.6	148
250	5/5	26.4	27.3	0.9	110	3.8	210
400	5/5	26.9	24.9	-2.0	100	4.6	370
650	5/5	27.4	26.2	-1.2	105	4.3	627

TABLE 12 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F, Mice in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (mg/kg)	Survival	Mean Body Weight (grams) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
FEMALE							
2-Methoxyethanol							
0	5/5	20.3	20.6	0.3		8.1	
200	5/5	21.0	19.6	-1.4	95	6.9	255
400	5/5	21.5	21.7	0.2	105	7.7	544
600	5/5	21.0	20.9	-0.1	101	9.2	971
1000	5/5	21.3	22.0	0.7	107	6.0	1094
1200	5/5	21.2	22.5	1.3	109	4.9	1124
2-Ethoxyethanol							
0	5/5	18.3	19.4	1.1		8.7	
300	5/5	18.9	19.6	0.7	101	8.0	403
600	5/5	19.1	19.7	0.6	102	7.1	793
900	5/5	18.6	20.2	1.6	104	6.6	1069
1500	5/5	18.5	20.0	1.5	103	6.9	1966
2500	5/5	18.7	20.3	1.6	105	5.8	2815
2-Butoxyethanol							
0	5/5	20.5	20.4	-0.1		8.3	
100	5/5	20.8	20.9	0.1	102	6.1	150
150	5/5	20.5	20.4	-0.1	100	6.0	237
250	5/5	20.6	20.8	0.2	102	6.3	406
400	5/5	20.7	20.7	0.0	101	6.6	673
650	5/5	20.7	19.3	-1.4	95	8.9	1364

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) x 100.
[4] Day of death: 10.

The only clinical observation noted for male mice treated with 2-methoxyethanol was dehydration in 2 of 5 males in the 1200 mg/kg group. Female mice receiving 2-methoxyethanol at dose levels of 0 (1/5 mice), 600 (2/5 mice), 1000 (1/5 mice), or 1200 mg/kg (1/5 mice) were found dehydrated. In the 2-ethoxyethanol study, 1 male in the 900 mg/kg group that died was hypoactive and dehydrated prior to death. No other clinical signs of toxicity were reported in mice treated with 2-ethoxyethanol. In the 2-week study of 2-butoxyethanol, 3 of 5 males in the 400 mg/kg group and 2 of 5 males in the 650 mg/kg group were dehydrated. Dehydration was also noted for 1 female treated with 400 mg/kg and 3 females treated with 650 mg/kg. One female receiving 650 mg/kg 2-butoxyethanol was thin on Day 14 and hunched and moribund on Day 15.

2-Methoxyethanol: In the 2-week study of 2-methoxyethanol in mice, changes in organ weights were minimal. For male mice, absolute and relative testis and thymus weights decreased in a dose-related fashion, and for female mice in the 2 highest dose groups (1000 and 1200 mg/kg), absolute and relative thymus weights were lower than those of the control group.

2-Ethoxyethanol: As with the 2-methoxyethanol study, changes in organ weights for mice in the 2-week study of 2-ethoxyethanol were minimal. For males in the high-dose (2500 mg/kg) group, relative testis weight was significantly lower than that of the control group. Slight but not significant decreases were also noted for the absolute testis weight of high-dose males and the absolute thymus weight of high-dose females.

2-Butoxyethanol: For male mice treated with 400 or 650 mg/kg 2-butoxyethanol for 2 weeks, absolute and relative thymus weights were marginally lower than the control values. No significant changes in organ weights were noted for females treated with 2-butoxyethanol.

No chemical-related gross lesions were noted in male or female mice in the 2-week study of 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol; microscopic evaluation of tissues was not performed.

For the 13-week studies of the ethylene glycol ethers in mice, chemical administration was changed from a mg/kg basis to a constant ppm in the drinking water. The maximum doses chosen for 2-methoxyethanol and 2-butoxyethanol were approximately equal to the doses that caused a measurable decrease in water consumption in the 2-week studies. The highest dose chosen for 2-butoxyethanol was inadvertently set about 4-fold higher than the appropriate high dose based on the 2-week study data, although this high dose did not result in mortality or marked toxicity.

13-Week Studies in B6C3F$_1$ Mice

No male or female mice receiving 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol died or were killed before the end of the studies. The mean body weight gain of male mice receiving 10,000 ppm 2-methoxyethanol and females receiving 8000 or 10,000 ppm 2-methoxyethanol were notably lower than control values. For male and female mice receiving 20,000 or 40,000 ppm 2-ethoxyethanol, body weight gains were notably lower than values for the control groups. Male and female mice receiving 3000 to 6000 ppm 2-butoxyethanol had slightly lower mean body weight gains relative to the control groups (Table 13; Figures 7-9).

In the 13-week study of ethylene glycol ethers, average water consumption was generally decreased in male and female mice in the 3 highest dose groups; in the 2 low-dose groups for each of the glycol ethers, water consumption amounts were, with a few exceptions, greater than or similar to those of the respective control groups (Table 13). Average compound consumption increased with dose for male and female mice treated with the glycol ethers (Table 13).

There were no significant clinical observations in male or female mice during the 13-week studies of 2-methoxyethanol and 2-butoxyethanol. The only treatment related clinical sign of toxicity noted for mice treated with 2-ethoxyethanol was emaciation for males and females in the 20,000 and 40,000 ppm groups.

TABLE 13 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F, Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
MALE							
2-Methoxyethanol							
0	10/10	24.0	39.3	15.3		4.5	
2000	10/10	24.0	40.2	16.2	102	4.9	295
4000	10/10	24.8	41.2	16.4	105	4.5	529
6000	10/10	24.4	38.1	13.7	97	4.1	765
8000	10/10	25.2	38.0	12.8	97	4.0	992
10,000	10/10	24.5	30.5	6.0	78	3.9	1367
2-Ethoxyethanol							
0	10/10	22.7	39.2	16.5		6.7	
2500	10/10	23.7	41.7	18.0	106	7.6	587
5000	10/10	23.5	43.1	19.6	110	6.5	971
10,000	10/10	22.8	41.0	18.2	105	6.3	2003
20,000	10/10	23.4	33.2	9.8	85	7.8	5123
40,000	10/10	23.9	32.5	8.6	83	5.2	7284
2-Butoxyethanol							
0	10/10	24.7	40.9	16.2		5.1	
750	10/10	24.9	40.0	15.1	98	5.2	118
1500	10/10	24.5	40.5	16.0	99	4.9	223
3000	10/10	24.8	38.0	13.2	93	6.0	553
4500	10/10	24.7	39.0	14.3	95	4.8	676
6000	10/10	24.5	38.2	13.7	93	3.7	694

TABLE 13 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (ppm)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	10/10	19.9	30.7	10.8		6.3	
2000	10/10	19.1	30.6	11.5	100	6.4	492
4000	10/10	19.8	30.4	10.6	99	5.8	902
6000	10/10	19.6	29.3	9.7	95	5.1	1194
8000	10/10	20.0	27.2	7.2	89	4.7	1489
10,000	10/10	20.4	24.9	4.5	81	4.5	1839
2-Ethoxyethanol							
0	10/10	19.3	32.0	12.7		8.7	
2500	10/10	19.0	34.0	15.0	106	7.5	722
5000	10/10	18.9	34.1	15.2	107	6.9	1304
10,000	10/10	19.1	30.2	11.1	94	6.9	2725
20,000	10/10	19.1	26.4	7.3	83	8.7	7255
40,000	10/10	19.0	24.9	5.9	78	6.1	11,172
2-Butoxyethanol							
0	10/10	20.1	31.6	11.5		6.2	
750	10/10	20.3	31.9	11.6	101	6.6	185
1500	10/10	20.2	30.8	10.6	97	6.5	370
3000	10/10	20.0	28.5	8.5	90	5.6	676
4500	10/10	19.9	29.7	9.8	94	4.8	861
6000	10/10	20.0	29.0	9.0	92	5.6	1306

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change of the animals in each dose group surviving to Week 13.
[3] (Dosed group mean/control group mean) x 100.

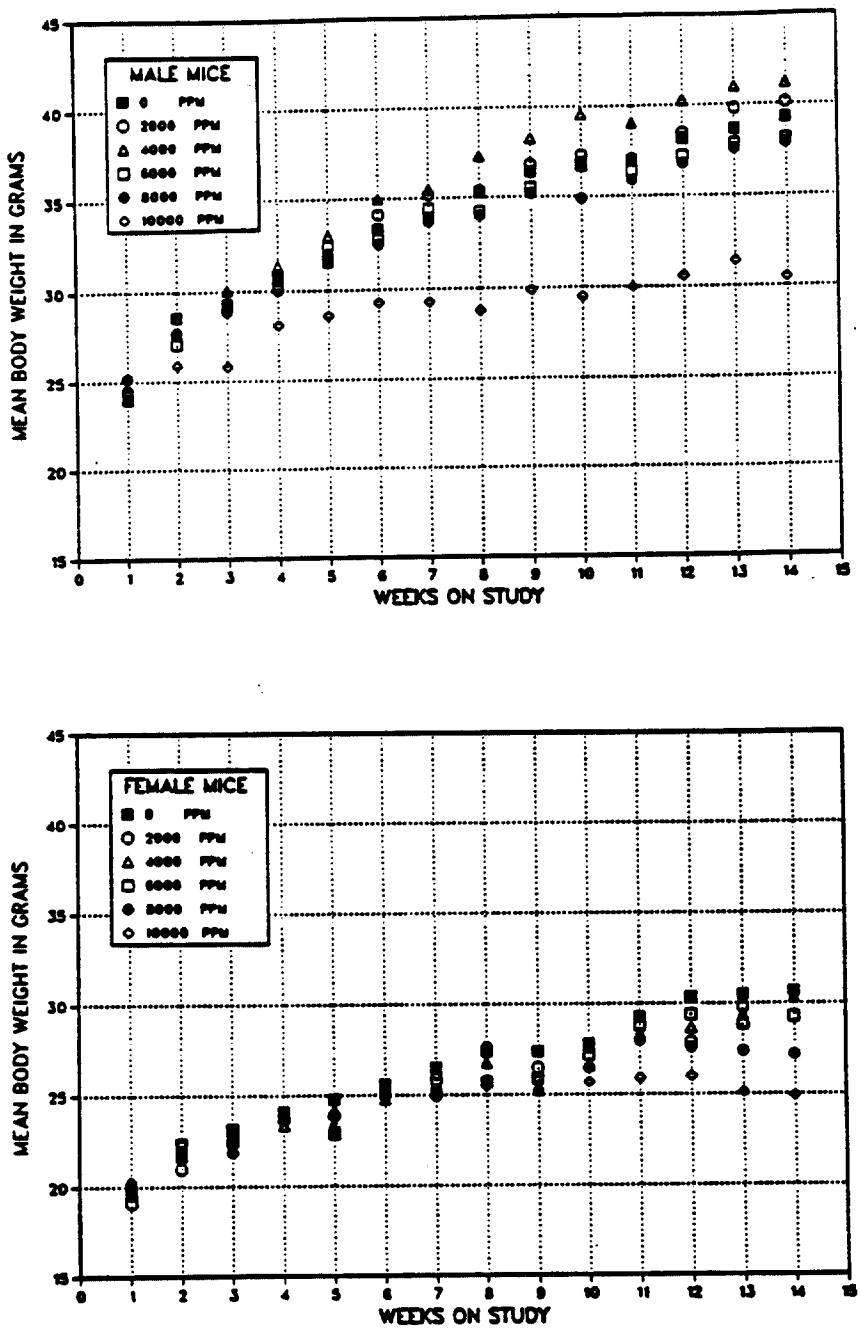

FIGURE 7 **Body Weights of B6C3F$_1$ Mice Administered 2-Methoxyethanol in Drinking Water for 13 Weeks**

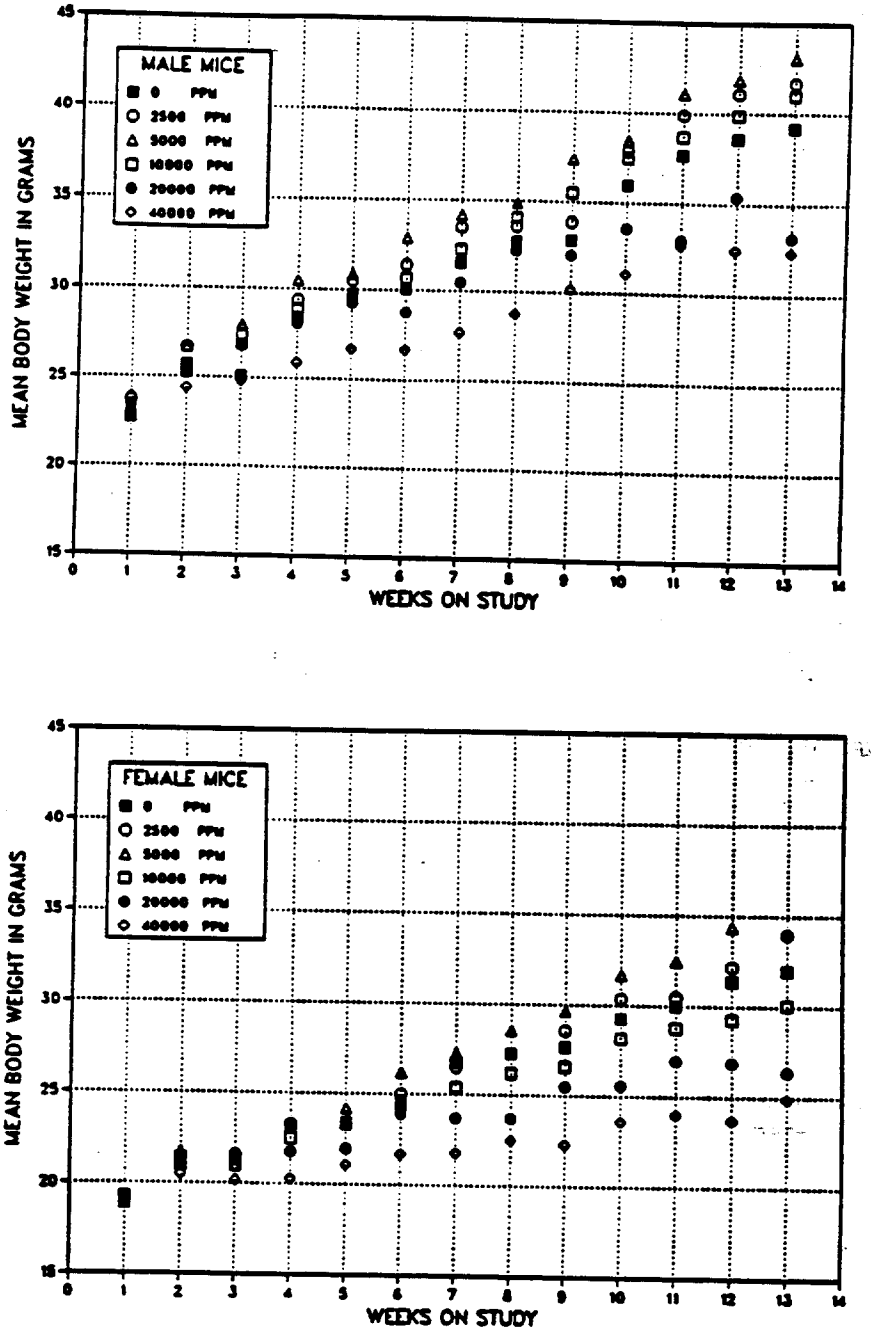

FIGURE 8 Body Weights of B6C3F$_1$ Mice Administered 2-Ethoxyethanol in Drinking Water for 13 Weeks

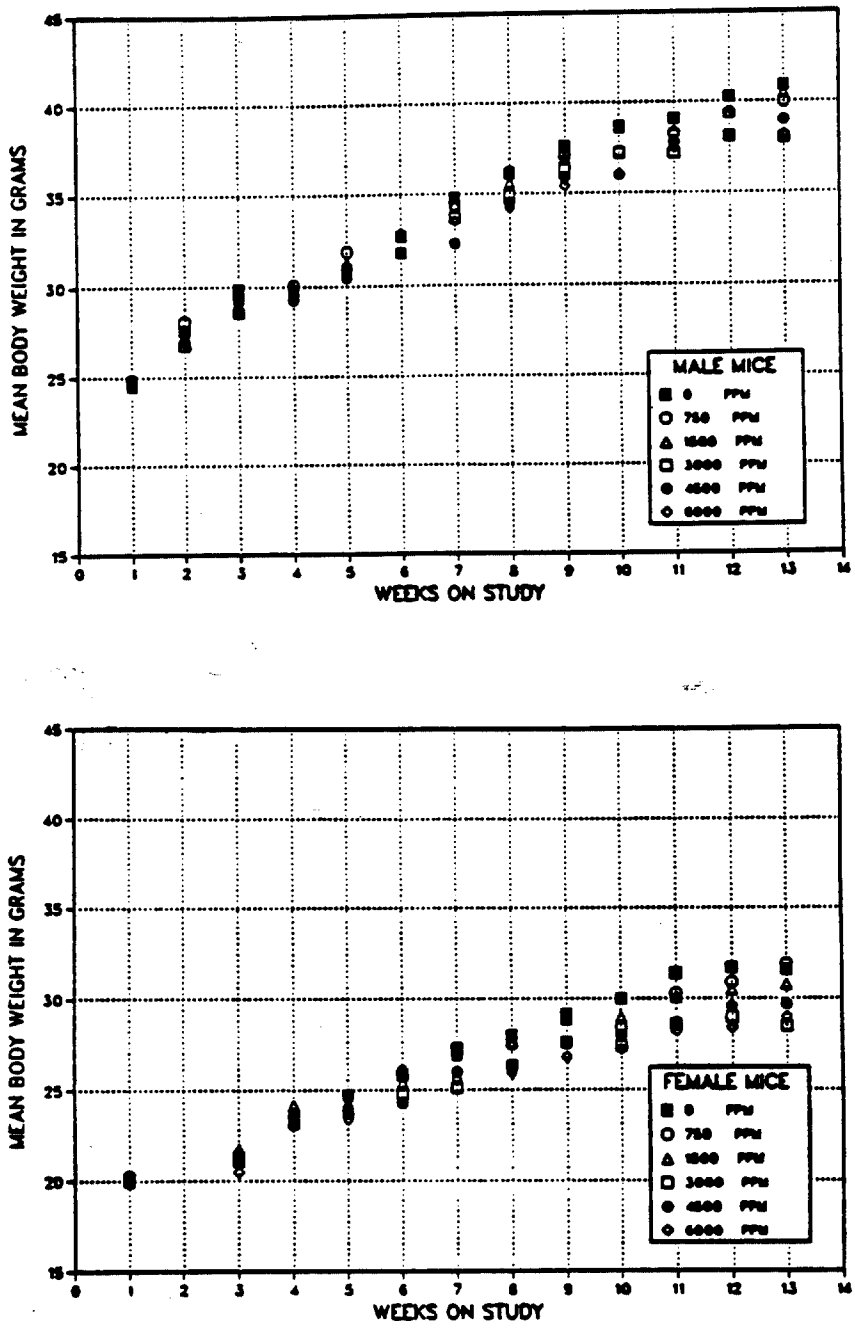

FIGURE 9 Body Weights of B6C3F, Mice Administered 2-Butoxyethanol in Drinking Water for 13 Weeks

2-Methoxyethanol: With the exception of decreases in thymus and testis weights, most changes in absolute and relative organ weights in the 13-week study of 2-methoxyethanol in mice could be attributed to low final mean body weights. Dose-related decreases were noted for the absolute and relative testis weights of male mice and the absolute and relative thymus weights of male and female mice (Table 14). Complete organ weight data for mice in the 13-week study of 2-methoxyethanol are provided in Appendix C, Tables C3 and C4.

TABLE 14 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	2000	4000	6000	8000	10,000
MALE						
n	10	10	10	10	9	10
Necropsy body wt	39.2	39.6	40.8	37.8	37.1	30.1**
Right testis						
Absolute	0.121	0.120	0.102**	0.029**	0.026**	0.023**
Relative	3.11	3.04	2.51**	0.77**	0.69**	0.76**
Thymus						
Absolute	0.046	0.047	0.047	0.039	0.036*	0.023**
Relative	1.17	1.18	1.15	1.04	0.98*	0.76**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.7	29.3	29.6	27.2	26.0**	23.9**
Thymus						
Absolute	0.048	0.055	0.049	0.042	0.037*	0.026**
Relative	1.63	1.89	1.67	1.57	1.46	1.09*

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

In the 13-week study of 2-methoxyethanol, chemical-related gross lesions were identified in the testis and thymus. Testes from mice in the 6000, 8000, and 10,000 ppm groups were small. The thymuses of males in the 8000 and 10,000 ppm groups and females in

the 10,000 ppm (high-dose) group were also smaller than those of the control animals. In male mice, degeneration of the testis was characterized microscopically by a dose-related, minimal to marked degeneration of the germinal epithelium in seminiferous tubules (Table 15); at the higher doses, the lumen of many tubules contained only Sertoli cells (Plate 9). In the thymus of most males from the 2 highest dose groups and females in the high-dose group, there was minimal to mild lymphoid depletion (atrophy) consisting of a reduction in the thickness of the thymic cortex and in the number of thymocytes.

TABLE 15 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	2000	4000	6000	8000	10,000
MALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	10 (1.0)	9 (1.0)	9 (1.0)	10 (1.1)
Thymus						
Atrophy	0[2]	–[3]	–	0	6 (1.5)	9 (2.0)
Testes						
Degeneration	0	0[2]	3 (1.0)	10 (3.0)	10 (4.0)	10 (4.0)
FEMALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	5 (1.0)	10 (1.0)	8 (1.1)	9 (1.0)	10 (1.0)
Thymus						
Atrophy	0[2]	–	–	–	0	4 (2.3)
Adrenal gland						
X-zone, hypertrophy	0	10 (2.1)	9[2] (2.9)	10 (3.1)	10 (3.7)	10 (3.8)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] n=9.
[3] Not applicable; tissue not examined for animals in this dose group.

Histopathologic changes were also present in the spleen of male and female mice and in the adrenal gland of female mice (Table 15). Increased hematopoiesis was present in the spleen of mice from all dosed groups, excluding male mice in the lowest dose group (2000 ppm), and was characterized by a marked increase in the number of megakaryocytes present in the red pulp (Plates 10-12). In the adrenal gland of female mice in all dosed

groups, there was hypertrophy of the X-zone. In dosed mice, there was a marked increase in the lipid vacuolization normally present in this region of the adrenal gland in young female mice (Plates 13-16).

A summary of lesions in mice treated with 2-methoxyethanol for 13-weeks is presented in Appendix B, Tables B1 and B2.

Sperm morphology evaluations were performed on male mice treated with 0, 2000, 4000, or 6000 ppm 2-methoxyethanol. Vaginal cytology evaluations were performed on female mice treated with 0, 6000, 8000, or 10,000 ppm 2-methoxyethanol. Results showed significant decreases in epididymal and cauda epididymal weights for males in the 6000 ppm group and in testicular weight for males in the 4000 and 6000 ppm groups (Appendix E, Table E7). The values for sperm motility were significantly less than controls for the 2000 and 6000 ppm groups, as were sperm concentration measurements for males treated with 2000 to 6000 ppm 2-methoxyethanol. Spermatid measurements were significantly lower than controls for males receiving 4000 or 6000 ppm 2-methoxyethanol. For females, all dose groups differed significantly from controls in the relative frequency of time spent in estrous stages (Appendix E, Table E8).

2-Ethoxyethanol: Most changes in absolute and relative organ weights in the 13-week study of 2-ethoxyethanol in mice could be attributed to low final mean body weights, excluding decreases in testis weights. Absolute testis weights were significantly decreased for males in the 2 highest dose groups (20,000 and 40,000 ppm) (Table 16). Complete organ weight data for mice in the 13-week study of 2-ethoxyethanol are provided in Appendix C, Tables C3 and C4.

TABLE 16 Testis Weights and Testis-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	2500	5000	10,000	20,000	40,000
n	10	10	10	10	10	10
Necropsy body wt	38.9	40.9	43.0	40.5	33.6*	31.9**
Right testis						
Absolute	0.119	0.124	0.123	0.119	0.097**	0.019**
Relative	3.06	3.05	2.86	2.95	2.88	0.59**

[1] Testis weights and body weights are given in grams; testis-weight-to-body-weight ratios are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

In the 13-week study of 2-ethoxyethanol, chemical-related gross lesions consisted of small testes and epididymides in mice from the 40,000 ppm group. Histopathologic changes were present in the spleen and testis of male mice and the spleen and adrenal gland of female mice (Table 17). In male mice, degeneration of the testis was characterized as a marked, diffuse loss of germinal epithelium in the seminiferous tubules. Histopathologic changes were not seen in the testis of mice in the lower dose groups. In the spleen of female mice in the 20,000 ppm group and male and females from the 40,000 ppm groups, there was a minimal to mild increase in hematopoiesis; there was also a minimal increase in splenic hematopoiesis in 1 female mouse in the 10,000 ppm group. Splenic hematopoiesis was characterized by an increase in the number of erythroid elements and megakaryocytes and was similar to that seen in mice from the 2-methoxyethanol study. Based upon histologic sections, there was no apparent effect in the bone marrow. In the adrenal gland, hypertrophy of the X-zone was present in all dose groups and was morphologically identical to that described for mice in the 2-methoxyethanol study.

A summary of lesions in mice treated with 2-ethoxyethanol for 13-weeks is presented in Appendix B, Tables B3 and B4.

TABLE 17 Incidence and Severity of Selected Histopathologic Lesions In B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	2500	5000	10,000	20,000	40,000
MALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	0	0	0	10 (1.6)
Testes						
Degeneration	0	0	0	0	0	10 (4.0)
FEMALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	0	1 (1.0)	9 (1.3)	10 (1.8)
Adrenal gland						
X-zone, hypertrophy	0	0	1 (2.0)	8 (1.8)	10 (2.8)	9 (2.4)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

Sperm morphology and vaginal cytology evaluations were performed on mice treated with 0, 5000, 10,000, or 20,000 ppm 2-ethoxyethanol. Epididymal and testicular weights were significantly lower than control values for males in the high-dose group (20,000 ppm) (Appendix E, Table E9). Values for sperm motility, spermatid heads per testis, and spermatid count were significantly lower than control values for males receiving 20,000 ppm 2-ethoxyethanol. All treated females had significantly longer estrous cycles than did controls (Appendix E, Table E10).

2-Butoxyethanol: In the 13-week study of 2-butoxyethanol in mice, all changes in organ weights were considered to be secondary to reduced body weights. Complete organ weight data for mice in the 13-week study of 2-butoxyethanol are provided in Appendix C, Tables C3 and C4.

There were no chemical-related gross or microscopic lesions in male or female mice administered 2-butoxyethanol in the drinking water for 13 weeks. A summary of lesions

Genetic Toxicity

2-Ethoxyethanol (Zeiger *et al.*, 1985), 2-methoxyethanol, and 2-butoxyethanol (Zeiger *et al.*, 1992) were negative in *Salmonella typhimurium* mutation tests conducted with and without induced hamster and rat liver S9 (Tables G1-G3). Each of the 3 glycol ethers was tested up to the proscribed maximum dose of 10,000 µg/plate. In the mouse lymphoma L5178Y cell mutation assay, 2-ethoxyethanol was negative without S9 but was judged to be weakly positive in 2 of 3 trials conducted in the presence of induced rat liver S9 (Table G4). Neither of the other 2 glycol ethers was tested in this assay.

2-Ethoxyethanol (Galloway *et al.*, 1987) and 2-butoxyethanol gave contrasting results in tests of induction of chromosomal damage in Chinese hamster ovary (CHO) cells *in vitro*. 2-Ethoxyethanol induced sister chromatid exchanges (SCEs) in CHO cells at very high concentrations (3170 and 9510 µg/mL), with and without S9 (Table G5). It also induced chromosomal aberrations (Abs) in CHO cells, but only in the absence of S9 (Galloway *et al.*, 1987; Table G7). The concentrations which produced a positive response were, as in the SCE test, very high (6830 and 9510 µg/mL). Despite these high concentrations of 2-ethoxyethanol, no cell cycle delay was observed in treated cultures. In contrast, 2-butoxyethanol induced cell cycle delay but did not induce either SCEs (Table G6) or Abs (Table G8) in CHO cells, with or without S9. In the Abs test without S9, a weakly positive response was obtained in the second trial at the highest dose tested (5000 µg/mL), but this response was not repeated in a third trial and the test was concluded to be negative. Because of the cell cycle delay caused by 2-butoxyethanol in the trials conducted without S9, a delayed harvest was used to increase the number of cells available for analysis.

2-Ethoxyethanol was the only 1 of the 3 glycol ethers to be tested for induction of sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* (Valencia *et al.*, 1985; Mason *et al.*, 1992; Table G9). Two separate experiments were performed using both feeding and injection as the route of administration; all results were negative.

in mice treated with 2-butoxyethanol for 13-weeks is presented in Appendix B, Tables B5 and B6.

Sperm morphology and vaginal cytology evaluations were performed for mice treated with 0, 3000, 4500, or 6000 ppm 2-butoxyethanol. All treated males had significantly lower testicular weights than control males (Appendix E, Table E11). Sperm motility values were also significantly lower than control values for all treated males. For treated females, estrous cycle lengths and percent of time spent in estrous stages were not significantly different from those of the control group (Appendix E, Table E12).

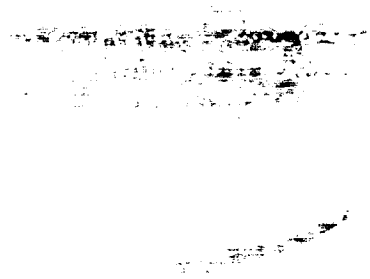

PLATE 1

Spleen of a male rat exposed to 3000 ppm 2-methoxyethanol showing marked thickening (fibrosis) of capsule (arrows) compared to the spleen from a control male rat shown in Plate 2. 128x.

PLATE 2

Spleen of a control male rat for comparison with Plates 1 and 3. Note typical appearance of thin fibrous capsule (arrows) compared to the spleen of a 2-methoxyethanol-treated rat in Plate 1. Scattered, darkly stained foci of hematopoiesis (asterisks) are present in addition to periarteriolar lymphoid sheath (L). Compare to the increased hematopoiesis present in the spleen from a 2-ethoxyethanol-treated rat shown in Plate 3. 128x.

PLATE 3

Spleen of a male rat exposed to 10,000 ppm 2-ethoxyethanol showing a marked increase in darkly staining hematopoietic cells compared to the spleen from a control rat shown in Plate 2. 128x.

PLATE 4

Liver from a female rat exposed to 6000 ppm 2-butoxyethanol showing hepatocyte degeneration adjacent to a central vein (V). Note the scattered darkly stained hepatocytes which appear shrunken with angular cytoplasmic borders and a densely stained nucleus (arrows). 240x.

PLATE 5

Testis of a control male rat from the stop-exposure study at 60 days showing normal morphologic appearance of seminiferous tubules. GMA section, 64x.

PLATE 6

Higher magnification of the testis shown in Plate 5. Compare with Plate 8. GMA section, 320x.

PLATE 1

PLATE 2

PLATE 3

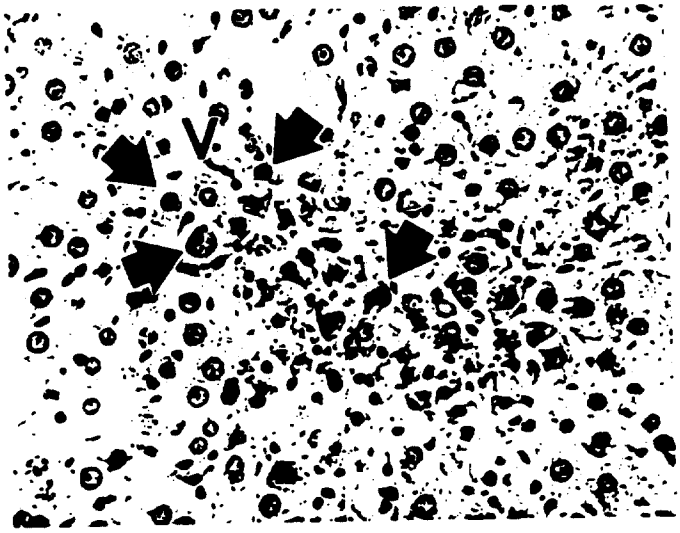

PLATE 4

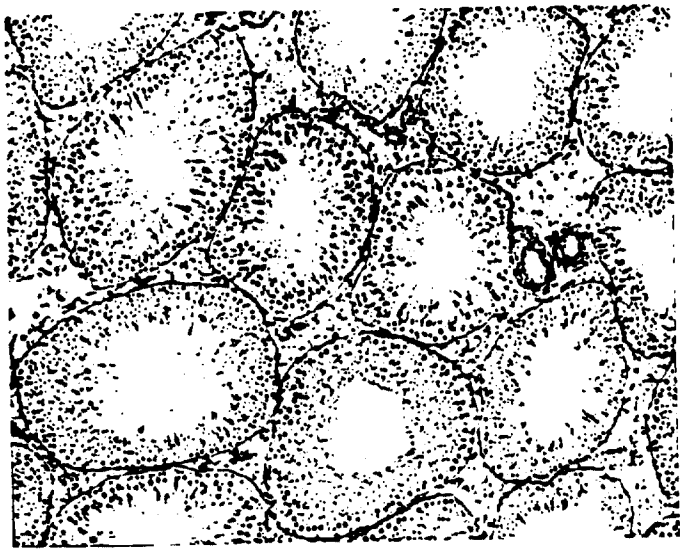

PLATE 5

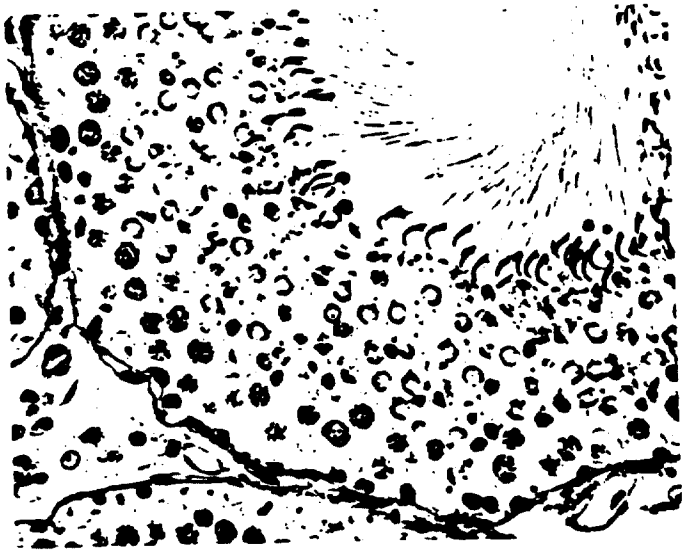

PLATE 6

PLATE 7

Testis of a male rat from the stop-exposure study exposed to 3000 ppm 2-methoxyethanol for 60 days with no recovery period. Note smaller diameter of the seminiferous tubules and marked depletion of spermatogenic cells. GMA section, 64x.

PLATE 8

Higher magnification of the testis shown in Plate 7. GMA section, 320x.

PLATE 9

Testis from a mouse exposed to 8000 ppm 2-methoxyethanol showing marked degeneration with no evidence of spermatogenesis in atrophic seminiferous tubules. 240x.

PLATE 10

Spleen from a control male mouse showing the normal appearance of the red pulp. Note the single megakaryocyte (arrow) in field. 160x.

PLATE 11

Spleen from a male mouse exposed to 10,000 ppm 2-methoxyethanol showing increased hematopoiesis characterized primarily by aggregates of megakaryocytes beneath the splenic capsule (arrows). 160x.

PLATE 12

Higher magnification of the mouse spleen in Plate 11 showing numerous multilobulated, sometimes darkly stained nuclei of megakaryocytes and foci of smaller darkly stained erythroid cell precursors. 240x.

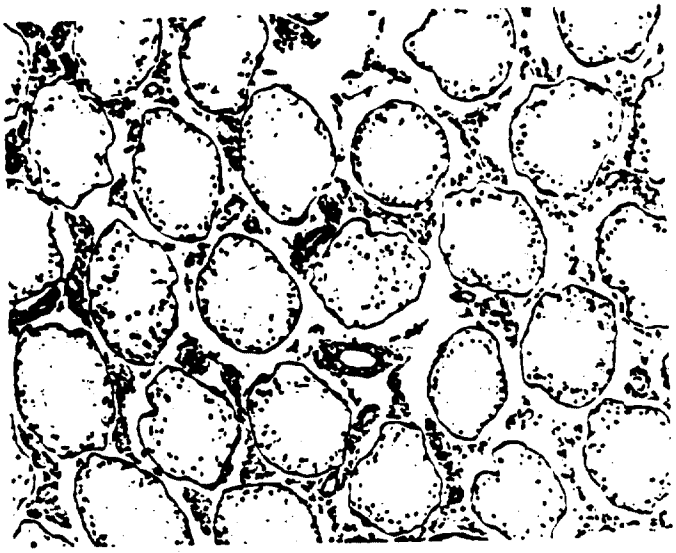

PLATE 7

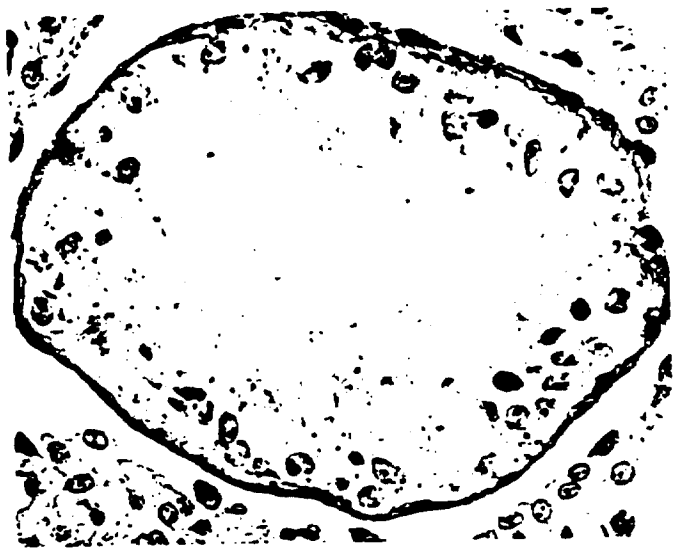

PLATE 8

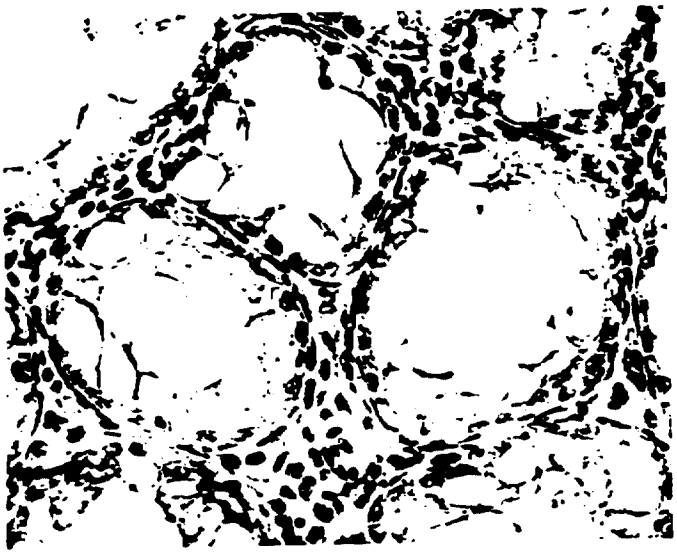

PLATE 9

PLATE 10

PLATE 11

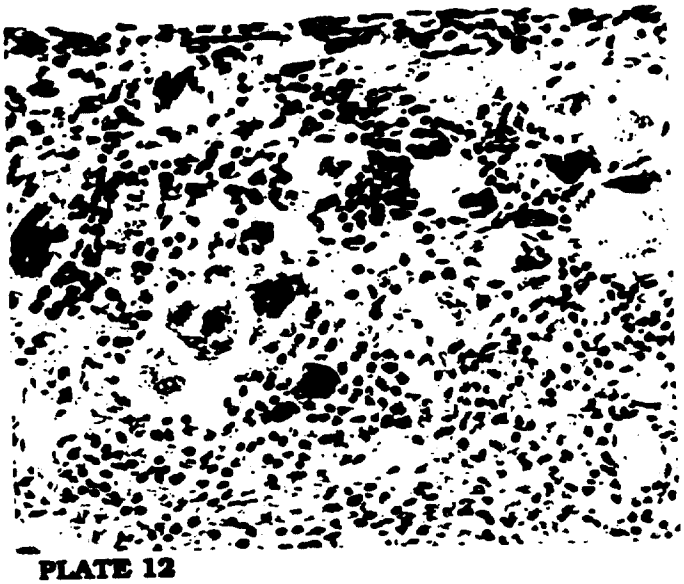

PLATE 12

PLATE 13

Adrenal gland from a control female mouse showing darkly stained X-zone between the pale staining adrenal cortex and the medulla (M). Note the scattered lipid vacuoles (arrows) present in this area. 30x.

PLATE 14

Higher magnification of the adrenal gland shown in Plate 13. 75x.

PLATE 15

Adrenal gland from a female mouse exposed to 10,000 ppm 2-methoxyethanol showing marked hypertrophy of the X-zone with slight compression of the cortex as a result of marked lipid vacuolization of the X-zone. 30x.

PLATE 16

Higher magnification of the adrenal gland shown in Plate 15. 75x.

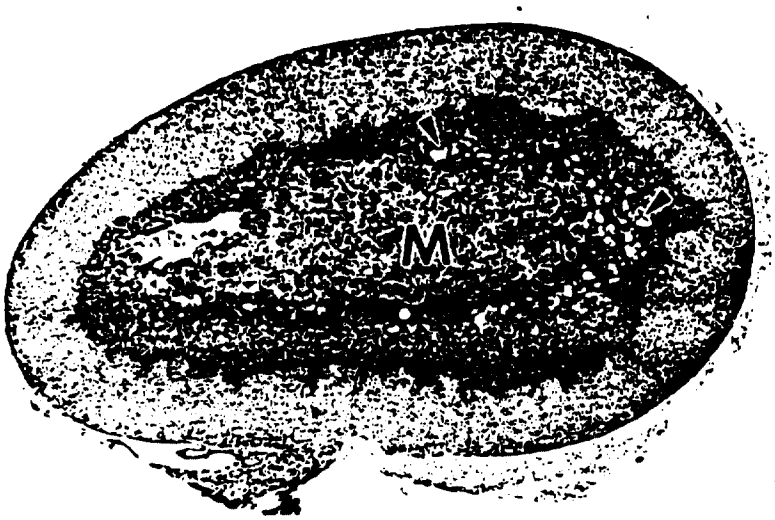

PLATE 13

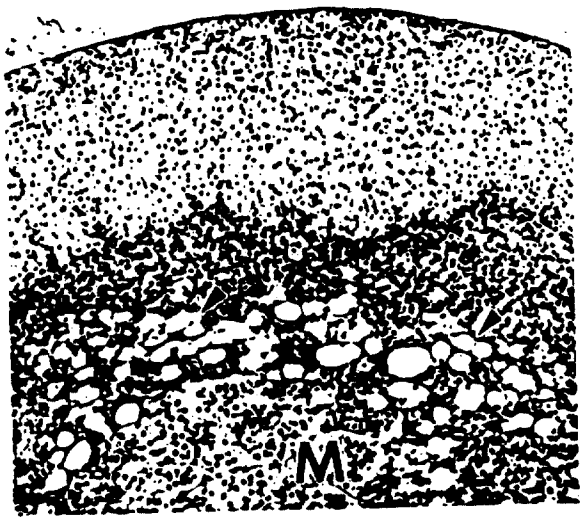

PLATE 14

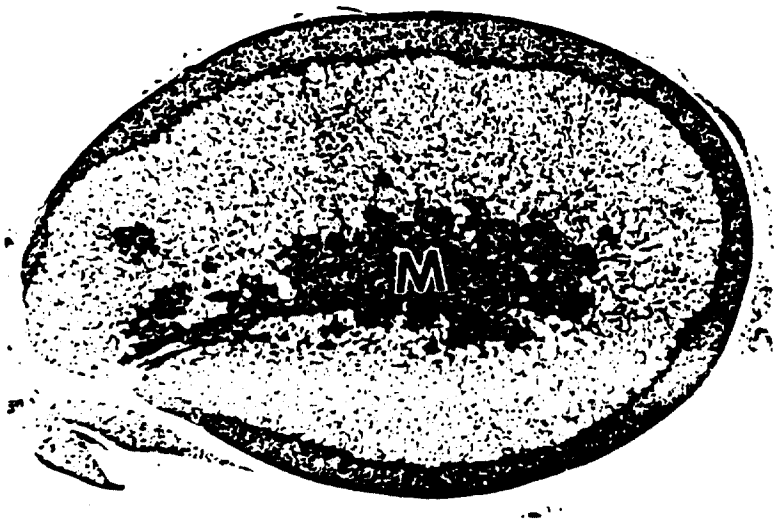

PLATE 15

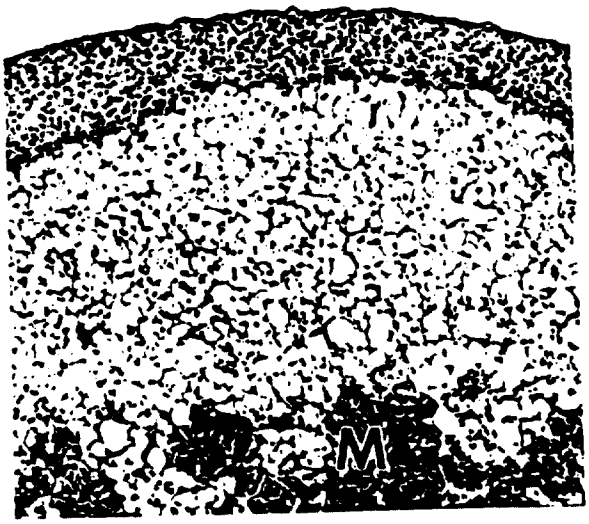

PLATE 16

DISCUSSION

The majority of the studies conducted with the glycol alkyl ethers have demonstrated that the toxic effects of these compounds result from their alkoxyacetic acid metabolites, and not as a direct response to the parent compounds. For example, developmental and reproductive toxicity similar to that caused by 2-methoxyethanol and 2-ethoxyethanol occurred when methoxyacetic acid and ethoxyacetic were administered alone (Miller *et al.*, 1982, 1983b; Foster *et al.*, 1987; Sleet *et al.*, 1988; Clarke *et al.*, 1991). Similarly, the hematoxicity of 2-butoxyethanol was affected by butoxyacetic acid; utilization of metabolic inhibitors of alcohol and aldehyde dehydrogenase in *in vivo* (Ghanayem *et al.*, 1987b, 1990a) and *in vitro* studies (Ghanayem *et al.*, 1989) clearly demonstrated that the alkoxyacetic acid metabolite was the effector of hemolysis.

The simplest hypothesis, then, for the difference in the toxicities of these compounds would seem to depend upon a variation in their mode or rate of metabolism to the respective alkoxyacetic acids. However, an examination of the metabolic data does not provide adequate information to explain all of the differences in the toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. Irrespective of the mode of administration (whether by the dermal, inhalation, or oral routes), alkoxyacetic acids or their derivatives were the major metabolites formed from the ethylene glycol ethers (Ghanayem *et al.*, 1987a; Medinsky *et al.*, 1990; Sabourin *et al.*, 1992a,b). For example, a study in F344 rats in which comparable doses of ^{14}C-labeled 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were administered for 24 hours in drinking water indicated that the majority of the ^{14}C from each of the chemicals was excreted in the urine or exhaled as CO_2, with less than 5% exhaled as unmetabolized glycol ether (Medinsky *et al.*, 1990). The metabolism of the glycol alkyl ethers was dependent upon chain length and, to a lesser extent, upon dose, such that the urinary alkoxyacetic acids excreted constituted 34% of the dose for 2-methoxyethanol, 25% to 40% of the dose for 2-ethoxyethanol, and 50% to 60% of the dose for 2-butoxyethanol; 10% to 30% of the dose for 2-methoxyethanol, 20% of the dose for 2-ethoxyethanol, and 8% to 10% of the dose for 2-butoxyethanol were eliminated in the urine as CO_2. Ethylene glycol was also excreted in the urine at 21%, 18%, and 10% of the doses for 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol, respectively (Medinsky *et al.*, 1990). There was no comparable

formation of ethylene glycol in rats administered glycol alkyl ethers by the dermal (Sabourin *et al.*, 1992b) or inhalation (Sabourin *et al.*, 1992a) routes, but the alkoxyacetic acids remained the major metabolic products.

It appears that ingestion of the glycol alkyl ethers enables dealkylation of a significant and varying percentage of the compounds prior to oxidation to the more toxic alkoxyacetic acid metabolites. This alternative pathway of metabolism, decreasing the formation of the more toxic alkoxyacetic acids, was inversely proportional to chain length and might partially account for the rank order of increasing toxicity demonstrated in the present studies (*e.g.*, 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol). Other factors in the comparative metabolism of the 3 glycol ethers that may contribute to this general rank order of toxicity were demonstrated in dermal studies in F344 rats; in these studies, although alkoxyacetic acids were the major urinary metabolite for all 3 compounds, only 2-butoxyethanol was metabolized to detectable amounts of a glucuronide conjugate (Sabourin *et al.*, 1992b). In addition, the proportion of the glycol ether metabolized to ethylene glycol from 2-ethoxyethanol was shown to exceed that metabolized from 2-methoxyethanol. Perhaps more importantly, a unique product of 2-methoxyethanol metabolism was identified that amounted to 30% to 50% of the doses administered. This unknown metabolite was identified as one with an HPLC retention time of 6 minutes, and it was a major metabolite of 2-methoxyethanol and was produced in amounts comparable to methoxyacetic acid. By comparison, there was 5.7% to 9.1% of this unknown metabolite in the urine of 2-ethoxyethanol-treated rats and none in the urine of 2-butoxyethanol-treated rats (Sabourin *et al.*, 1992b). It was shown that this unknown metabolite did not cochromatograph with glycolic acid, glyoxylic acid, or oxalic acid, all of which are possible metabolites of ethylene glycol. A gavage study in F344 rats identified a third metabolite, the sulfate conjugate of 2-butoxyethanol, which was present in the urine of animals dosed with 125 mg/kg 2-butoxyethanol but not in the urine of those dosed with 500 mg/kg (Ghanayem *et al.*, 1987a). Inhalation studies of 2-butoxyethanol in F344 rats indicated that formation of the alkoxyacetic acid metabolite was linearly related to exposure concentration up to doses that were toxic (Sabourin *et al.*, 1992a). These data are consistent with the toxicokinetic data from Ghanayem *et al.* (1990a), which showed blood levels of butoxyacetic acid were linearly related to the doses of 2-butoxyethanol administered. Thus, 2-butoxyethanol can be metabolized to butoxyacetic acid, the

glucuronide conjugate of 2-butoxyethanol, and the sulfate conjugate of 2-butoxyethanol, whereas the minor metabolic products of 2-methoxyethanol and 2-ethoxyethanol are different.

In the 13-week study of 2-butoxyethanol in rats, liver lesions were noted that may have been associated with the unique production of the glucuronide and sulfate conjugates of 2-butoxyethanol. Morphologic evidence of a chemical-related hepatotoxic effect was seen only in rats dosed with 2-butoxyethanol for 13 weeks. In this study, degeneration of hepatocytes and/or accumulation of pigment in Kupffer cell cytoplasm were observed in the livers of male and female rats in the 3 highest dose groups (3000, 4500, and 6000 ppm). However, evidence of a minimal effect in the staining appearance of cytoplasm (cytoplasmic alteration) was seen in almost half of the rats at the lowest administered dose of 2-butoxyethanol (750 ppm). Although this cytoplasmic alteration may have been the result of enzyme induction, there was no evidence of hepatocellular hypertrophy or increased liver weights in rats dosed with 2-butoxyethanol.

Hypertrophy of the X-zone, a rare lesion, occurred in the endocrine system of female mice treated with 2-methoxyethanol or 2-ethoxyethanol for 13 weeks. This change was most prominent in the 2-methoxyethanol study, where a no-effect level was not achieved. The X-zone is a portion of the adrenal gland between the medulla and outer cortex of female mice that normally undergoes an age-related degeneration and atrophy (Dunn, 1970). Typically associated with this atrophy is a variable amount of lipid vacuolization in the X-zone. Experimentally, the X-zone degeneration and atrophy have been shown to occur more rapidly with dietary restriction or with the administration of chemicals or corticosteroid hormones (Dunn, 1970). In the present studies, hypertrophy of the X-zone in treated mice was the result of a marked lipid vacuolization rather than a chemical-related enhancement of the spontaneously occurring atrophy. Similar findings have also been reported in female mice treated with other compounds, including thyroxine and methanol (Ribelin, 1984).

Perhaps because of the differences in the metabolism of the 3 glycol alkyl ethers, their principal target organs of toxicity also vary somewhat. 2-Methoxyethanol is primarily a reproductive and developmental toxicant, inducing spermatotoxicity and teratogenicity, as

is 2-ethoxyethanol to a lesser extent (NIOSH, 1991). 2-Butoxyethanol is primarily a hematotoxic agent in the erythrocyte series (NIOSH, 1990), while 2-methoxyethanol and 2-ethoxyethanol act primarily as hematotoxic agents in the leukocyte series (NIOSH, 1991). The overlap in their toxicities depends primarily upon dosage.

Comparable absolute or molar equivalent doses were not used in the present studies, but a general barometer to approximate the comparative toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol was provided by an examination of the data for thymus-weight-to-body-weight ratios. In the 2-week studies, decreases in thymus weight occurred at all doses of 2-methoxyethanol and 2-ethoxyethanol in male and female rats, as well as at the highest dose of 2-butoxyethanol administered to female rats. Male and female mice treated with the ethylene glycol ethers for 2 weeks also exhibited decreases in thymus weight. In the 13-week studies, thymic atrophy was greater in rats administered 2-methoxyethanol than in those given 2-ethoxyethanol, and it was much less severe in rats receiving 2-butoxyethanol. Similar responses in thymic weight reflected the comparative toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol in mice treated for 13 weeks.

The rank order of testicular toxicity in male rats and mice was analogous to that demonstrated in the thymus. Decreases in testicular and epididymal weights were greatest in animals treated with 2-methoxyethanol and were also significant in animals treated with 2-ethoxyethanol; in animals receiving 2-butoxyethanol, decreases in testicular and epididymal weights were much less severe. Testicular atrophy was accompanied by lesions characterized by degeneration of the germinal epithelium in the seminiferous tubules of the testes, abnormal sperm morphology, and reduced sperm counts.

In a separate stop-exposure study conducted to determine the persistence of the testicular toxicity, it was evident that 2-methoxyethanol exerted a greater toxic effect than 2-ethoxyethanol. Rats treated with 1500 or 3000 ppm 2-methoxyethanol for 60 days had greater degeneration of the seminiferous tubules of the testes than did rats treated with 5000 ppm 2-ethoxyethanol for 60 days. 2-Methoxyethanol also caused persistent degenerative lesions after 30 and 56 days of recovery. At the 5000 ppm exposure level, 2-ethoxyethanol caused no initial lesions during the 60-day exposure period but elicited

minimal degeneration in the testes in most male rats at both time points in the recovery period. In the stop-exposure study of 2-butoxyethanol, there was no testicular toxicity in rats in any of the treatment groups during the exposure or recovery periods. Additional studies to investigate the mechanism of action of 2-methoxyethanol for spermatotoxicity suggested that calcium deregulation in testicular cells by 2-methoxyethanol may be directly or indirectly responsible for the toxicity. In these studies, 1, 2, 3, or 4 doses of the calcium channel blockers verapamil or diltiazem, given in combination with a single gavage dose of 300 mg/kg 2-methoxyethanol, partially prevented testicular damage in male F344 rats (Ghanayem and Chapin, 1990).

The teratogenicity of 2-methoxyethanol has been well characterized in a series of studies using the CD-1 mouse (Sleet *et al.*, 1988; Clarke *et al.*, 1991); abnormalities in cranial development and limb bud morphogenesis occurred after pregnant mice were exposed to 2-methoxyethanol on gestation Days 7 and 11, respectively. Subsequently, it was shown that teratogenesis correlated with peak concentrations of methoxyacetic acid in the blood of the dam, embryo, and surrounding extraembryonic fluids following subcutaneous administration of 250 mg/kg 2-methoxyethanol. The urinary elimination half-life of methoxyacetic acid from both maternal and embryonic compartments was determined to be 5 to 6 hours, and the embryonic exposure was estimated at 60 to 70 mmol/hr per kg (Clarke *et al.*, 1991). The pharmacokinetics of 2-methoxyethanol/methoxyacetic acid were similar in the pregnant rat, with a calculated half-life for methoxyacetic acid of about 12 hours (Scott *et al.*, 1989). By comparison, the half-life of methoxyacetic acid in pregnant macaque monkeys dosed with 12 to 36 mg/kg 2-methoxyethanol was about 20 hours, compared to a half-life of greater than 70 hours in humans (Groeseneken *et al.*, 1989).

It was evident that common cellular targets for glycol alkyl ether toxicity were undifferentiated, rapidly dividing cells, such as those that occur in the embryo or in the hematopoietic system of adult animals (Nagano *et al.*, 1981, 1984; Tyler, 1984). Because of this demonstrable cell sensitivity, studies were conducted to determine the potential anti-tumorigenicity of the glycol alkyl ethers. Previous toxicity studies showed that administration of 2-methoxyethanol or 2-ethoxyethanol resulted in a decrease in white blood cells and in bone marrow cellularity (Hong *et al.*, 1988, 1989). Initial studies demonstrated that 2-methoxyethanol and 2-ethoxyethanol prevented mortality in mice

challenged with L1210 leukemia cells in an allogeneic tumor model (Houchens *et al.*, 1984). Additional investigations with an F344 rat cellular tumor model showed that 2-ethoxyethanol would inhibit the progression of leukemia in syngeneic transplant recipients (Dieter *et al.*, 1989) and that the degree of inhibition was about one-half as effective as that from an equivalent dosage of 2-methoxyethanol (Dieter *et al.*, 1990). These findings were confirmed in the present independent study (Appendix F). Further, it was found that among 9 different glycol ethers, including 2-methoxyethanol, 2-ethoxyethanol, 2-butoxyethanol, ethylene glycol, and diethylene glycol, only 2-methoxyethanol and 2-ethoxyethanol inhibited the progression of leukemia (Dieter *et al.*, 1990).

Subsequent immunotoxicity investigations in Sprague-Dawley rats with 2-methoxyethanol and 2-butoxyethanol examined the relationship between thymic atrophy and immune parameters such as natural killer cell function, specific antibody production, splenocyte production of γ-interferon, and spleen cell counts (Exon *et al.*, 1991). The results of these investigations provide 1 possible explanation for the specificity of the anti-tumorigenic activity of 2-methoxyethanol and 2-ethoxyethanol. The paucity of immune effects from 2-butoxyethanol is also in concert with the hypothesis that the anti-leukemic effects of 2-methoxyethanol and 2-ethoxyethanol were a result of an indirect effect of the glycol ethers on host immune effectiveness. Male and female rats exposed to 1600 to 6000 ppm 2-methoxyethanol or 2-butoxyethanol in drinking water for 21 days exhibited the expected decreases in body weights and thymus and testis weights. There were dose-related increases in natural killer cell cytotoxic activity and decreases in specific antibody production in both sexes of rats treated with 2-methoxyethanol. Splenocyte production of γ-interferon was decreased in males exposed to 2000 or 6000 ppm 2-methoxyethanol and in females treated with the high dose. Spleen cell numbers were reduced in males given the high dose of 2-methoxyethanol and in both dose groups of female rats. One immune parameter was affected by 2-butoxyethanol treatment; natural killer cell activity was decreased in the low-dose groups but not in the high-dose groups of both sexes. These data provide further credibility for the anti-tumorigenic activity of 2-methoxyethanol and also provide 1 explanation for the unusual specificity exhibited by only 2 of the 11 glycol ether compounds investigated. The mode of action of 2-methoxyethanol, and to a lesser extent 2-ethoxyethanol, may be to effect an *in vivo* stimulation of tumoricidal activity

in the natural immune defense system of the host. It is unlikely that 2-methoxyethanol acts directly as a cytotoxic agent, based on, (1), data showing that the spermatotoxic and teratotoxic metabolite of 2-methoxyethanol, methoxyacetic acid, was ineffective in reducing the number of rodent leukemia cells after *in vitro* exposure (Dieter *et al.*, 1990) and, (2), the *in vivo* data from Houchens *et al.* (1984), which showed that B6C3F_1 mice given allogeneic L1210 tumor cells and treated with 2-methoxyethanol or 2-ethoxyethanol were protected from mortality while syngeneic CD2F_1 mice were not.

In the present studies, treatment with the ethylene glycol ethers produced 2 different hematologic profiles that are consistent with distinct mechanisms. 2-Methoxyethanol produced pancytopenia characterized by a poorly regenerative, normochromic, normocytic to microcytic anemia, leukopenia, and thrombocytopenia. These findings indicate a treatment-related effect at the level of a pluripotent stem cell or a disruption of the hematopoietic microenvironment necessary for maintenance of normal hematopoiesis. In contrast, the regenerative, macrocytic, normochromic to hypochromic anemias produced by 2-ethoxyethanol and 2-butoxyethanol are consistent with an appropriate response to hemolysis of circulating erythrocytes (RBCs) accompanied by cellular swelling.

In previous *in vivo* experiments with 2-butoxyethanol, swelling of circulating RBCs preceded the onset of intravascular hemolysis (Ghanayem *et al.*, 1990b). Incubation of RBCs with butoxyacetic acid, an active metabolite of 2-butoxyethanol, also produced swelling of the cells (increased hematocrit and mean cell volume) shortly before lysis occurred (Ghanayem *et al.*, 1992). At most time points during the current studies, anemias produced by treatment with 2-butoxyethanol and 2-ethoxyethanol were generally regenerative (increase in reticulocyte counts), macrocytic, and hypochromic (occasionally, normochromic). Therefore, in addition to macrocytosis related to increased numbers of reticulocytes (which would be normochromic), the hypochromic nature of these anemias (produced by an increase in cell size resulting in a decreased ratio of hemoglobin concentration to hematocrit) indicates that a component of this effect was produced by cellular swelling.

In summary, the rank order of toxicity for the 3 glycol alkyl ethers in these studies was 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol. Although the metabolism of the

3 chemicals was similar (resulting in production of their respective alkoxyacetic acids), dissimilar, minor metabolites were reported to be produced at different rates by each of the chemicals, and these minor metabolites may partially account for the specificity of the toxicity exerted by 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. The major target organs for toxicity were the testes in males of both species and the hematopoietic system in both sexes and species. 2-Methoxyethanol appeared to act primarily as a spermatotoxic and immunotoxic agent. 2-Ethoxyethanol was intermediate as a toxic agent and was only effective in the highest dose ranges, while 2-butoxyethanol was relatively nontoxic at the doses tested and only affected the erythroid series in the hematopoietic system.

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APPENDIX A

Summary of Nonneoplastic Lesions in Rats

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Early Deaths						
Natural Death					7	8
Moribund Sacrifice					1	2
Survivors						
Terminal Sacrifice	10	10	10	10	2	
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, cecum	(10)			(10)	(5)	(5)
Lymphoid tissue, hyperplasia, reticulum cell				1 (10%)		
Intestine small, ileum	(10)			(10)	(5)	(5)
Peyer's patch, hyperplasia, reticulum cell				1 (10%)		
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium						4 (40%)
Hepatodiaphragmatic nodule				2 (20%)	2 (20%)	
Necrosis					1 (10%)	2 (20%)
Pancreas	(10)			(10)	(9)	(10)
Acinus, atrophy					1 (11%)	
Pharynx					(4)	
Palate, bacterium					4 (100%)	
Palate, ulcer					4 (100%)	
Salivary glands	(10)			(10)	(9)	(10)
Atrophy					2 (22%)	10 (100%)
Stomach, glandular	(10)	(2)	(10)	(10)	(8)	(10)
Erosion					2 (25%)	6 (60%)
Mineralization					1 (13%)	2 (20%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium						1 (10%)
Cardiomyopathy	2 (20%)	2 (20%)	6 (60%)	1 (10%)	2 (22%)	2 (20%)
Endocrine System						
Adrenal gland, cortex	(10)			(10)	(9)	(10)
Bacterium					1 (11%)	
Hemorrhage					3 (33%)	8 (80%)
General Body System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Genital System						
Epididymis	(10)	(9)	(10)	(10)	(10)	(10)
Aspermia			10 (100%)	10 (100%)	9 (90%)	10 (100%)
Fat, inflammation, chronic active				1 (10%)		
Preputial gland	(10)	(10)	(10)	(10)	(10)	(8)
Atrophy				1 (10%)	9 (90%)	8 (100%)
Prostate	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					9 (90%)	10 (100%)
Inflammation, chronic active					2 (20%)	
Metaplasia, squamous					1 (10%)	
Seminal vesicle	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					8 (80%)	9 (90%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy		7 (70%)	10 (100%)	10 (100%)	9 (90%)	10 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular					8 (80%)	10 (100%)
Lymph node	(10)	(10)	(10)	(10)	(9)	(9)
Mediastinal, angiectasis				1 (10%)	1 (11%)	
Mediastinal, depletion lymphoid					1 (11%)	1 (11%)
Mediastinal, infiltration cellular, lymphocyte		1 (10%)				
Pancreatic, angiectasis		1 (10%)				
Lymph node, mandibular	(10)	(10)	(10)	(10)	(9)	(9)
Angiectasis		1 (10%)	1 (10%)			
Depletion lymphoid					9 (100%)	9 (100%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(7)	(9)
Angiectasis					2 (29%)	2 (22%)
Depletion lymphoid				1 (10%)	6 (86%)	9 (100%)
Spleen	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid					7 (78%)	10 (100%)
Capsule, fibrosis		1 (10%)	4 (40%)	10 (100%)	5 (56%)	1 (10%)
Thymus	(10)	(10)	(9)	(10)	(9)	(9)
Depletion lymphoid			3 (33%)	2 (20%)	9 (100%)	9 (100%)
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)		(2)	(10)	(9)	(10)
Metaphysis, atrophy					9 (100%)	10 (100%)
Skeletal muscle	(10)			(10)	(9)	(10)
Mineralization					1 (11%)	
Nervous System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium					1 (11%)	
Fungus						1 (10%)
Hemorrhage	1 (10%)	2 (20%)			1 (11%)	2 (20%)
Inflammation, chronic active	8 (80%)	6 (60%)	7 (70%)	9 (90%)	6 (67%)	2 (20%)
Mediastinum, bacterium						1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium					2 (22%)	2 (20%)
Infarct					1 (11%)	
Inflammation, acute					1 (11%)	
Cortex, mineralization					3 (33%)	1 (10%)
Papilla, mineralization						1 (10%)
Renal tubule, regeneration	6 (60%)	6 (60%)	5 (50%)			1 (10%)
Urinary bladder	(10)	(1)	(1)	(9)	(7)	(9)
Calculus gross observation		1 (100%)	1 (100%)			
Calculus micro observation only		1 (100%)	1 (100%)			
Artery, mineralization	1 (10%)					

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Early Deaths						
Moribund Sacrifice					3	6
Natural Death					2	4
Survivors						
Terminal Sacrifice	10	10	10	10	5	
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium						1 (10%)
Hepatodiaphragmatic nodule	3 (30%)	1 (10%)	3 (30%)		1 (10%)	1 (10%)
Inflammation, chronic active					1 (10%)	
Necrosis						1 (10%)
Mesentery	(1)					
Fat, necrosis	1 (100%)					
Pharynx					(1)	
Palate, bacterium					1 (100%)	
Palate, fungus					1 (100%)	
Palate, ulcer					1 (100%)	
Salivary glands	(10)			(10)	(10)	(10)
Atrophy					2 (20%)	8 (80%)
Stomach, forestomach	(10)			(10)	(10)	(10)
Hemorrhage						1 (10%)
Hyperplasia						1 (10%)
Stomach, glandular	(10)			(10)	(10)	(10)
Erosion					3 (30%)	2 (20%)
Mineralization						1 (10%)
Cardiovascular System						
Heart	(10)			(10)	(10)	(10)
Cardiomyopathy				1 (10%)		2 (20%)
Endocrine System						
Adrenal gland, cortex	(10)			(10)	(10)	(10)
Hemorrhage					3 (30%)	8 (80%)
Pituitary gland	(10)	(1)		(10)	(10)	(10)
Pars distalis, cyst		1 (100%)				
General Body System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Genital System						
Clitoral gland	(10)	(10)	(10)	(8)	(10)	(8)
Atrophy				4 (50%)	8 (80%)	8 (100%)
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				6 (60%)	10 (100%)	10 (100%)
Cyst	2 (20%)	4 (40%)	2 (20%)			
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				8 (80%)	9 (90%)	10 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular			1 (10%)	7 (70%)	6 (60%)	9 (90%)
Fibrosis, focal	2 (20%)	3 (30%)	5 (50%)	3 (30%)	1 (10%)	
Lymph node	(10)	(10)	(10)	(10)	(10)	(10)
Mediastinal, angiectasis						4 (40%)
Mediastinal, depletion lymphoid						5 (50%)
Pancreatic, angiectasis						1 (10%)
Pancreatic, infiltration cellular, histiocyte		1 (10%)				
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis						1 (10%)
Depletion lymphoid					2 (20%)	5 (50%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(9)
Angiectasis					2 (20%)	5 (56%)
Depletion lymphoid					5 (50%)	8 (89%)
Infiltration cellular, histiocyte			2 (20%)			
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid			1 (10%)	1 (10%)	5 (50%)	10 (100%)
Fibrosis						1 (10%)
Capsule, fibrosis			3 (30%)	5 (50%)		
Thymus	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid			1 (10%)	9 (90%)	7 (78%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)			(10)	(10)	(10)
Metaphysis, atrophy					10 (100%)	10 (100%)
Nervous System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Edema						1 (10%)
Fungus						1 (10%)
Hemorrhage		3 (30%)		1 (10%)		2 (20%)
Inflammation, chronic active	1 (10%)	6 (60%)	4 (40%)	4 (40%)	4 (40%)	3 (30%)
Nose	(10)			(10)	(10)	(10)
Inflammation, acute					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium					1 (10%)	
Inflammation, acute					1 (10%)	1 (10%)
Cortex, mineralization	10 (100%)	8 (80%)	8 (80%)	8 (80%)	9 (90%)	4 (40%)
Renal tubule, regeneration		4 (40%)	2 (20%)		2 (20%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Early Deaths						
Moribund Sacrifice						5
Survivors						
Terminal Sacrifice	10	10	10	10	10	
Aborted						5
Animals Examined Microscopically	10	10	10	10	10	5
Alimentary System						
Intestine large, cecum	(10)				(10)	(5)
Parasite metazoan	2 (20%)				1 (10%)	
Intestine large, colon	(10)				(10)	(5)
Parasite metazoan	2 (20%)					1 (20%)
Intestine large, rectum	(10)				(10)	(5)
Parasite metazoan	1 (10%)					1 (20%)
Intestine small, ileum	(10)			(2)	(10)	(5)
Hyperplasia, lymphoid				2 (100%)		
Intestine small, jejunum	(10)			(1)	(10)	(5)
Hyperplasia, lymphoid				1 (100%)		
Liver	(10)	(10)	(10)	(10)	(10)	(5)
Hematopoietic cell proliferation					9 (90%)	
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)	1 (10%)		
Inflammation, chronic, focal	1 (10%)				3 (30%)	
Hepatocyte, centrilobular, degeneration						5 (100%)
Kupffer cell, pigmentation					10 (100%)	5 (100%)
Pancreas	(10)	(1)			(10)	(5)
Acinus, atrophy	2 (20%)					
Pharynx						(1)
Palate, ulcer, acute, focal						1 (100%)
Salivary glands	(10)				(10)	(5)
Atrophy						5 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(5)
Edema, focal						1 (20%)
Hyperplasia, focal, squamous						1 (20%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(5)
Edema, focal						1 (20%)
Inflammation, focal						1 (20%)
Cardiovascular System						
Heart	(10)				(10)	(5)
Cardiomyopathy	10 (100%)				6 (60%)	
Inflammation, chronic, focal					1 (10%)	

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Endocrine System						
Pituitary gland	(10)				(10)	(5)
Pars distalis, cyst	1 (10%)				1 (10%)	
General Body System						
None						
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(5)
Aspermia					10 (100%)	5 (100%)
Granuloma sperm				1 (10%)		
Preputial gland	(9)	(10)	(10)	(10)	(10)	(5)
Atrophy					1 (10%)	5 (100%)
Cyst			1 (10%)	1 (10%)		
Dilatation	1 (11%)	1 (10%)				
Inflammation, chronic, focal	1 (11%)				1 (10%)	
Prostate	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy			6 (60%)	7 (70%)	10 (100%)	5 (100%)
Seminal vesicle	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy						4 (80%)
Testes	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy				10 (100%)	10 (100%)	5 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy						5 (100%)
Proliferation					10 (100%)	
Lymph node	(10)	(3)	(1)	(5)	(10)	(5)
Mediastinal, hemorrhage, acute	1 (10%)					
Pancreatic, hyperplasia, lymphoid				1 (20%)	2 (20%)	
Lymph node, mandibular	(10)	(2)	(1)	(4)	(10)	(5)
Atrophy						3 (60%)
Congestion		1 (50%)	1 (100%)	3 (75%)		
Hyperplasia, lymphoid			1 (100%)	1 (25%)		
Lymph node, mesenteric	(10)	(1)			(10)	(5)
Atrophy						5 (100%)
Pigmentation						1 (20%)
Spleen	(10)	(10)	(10)	(10)	(10)	(5)
Pigmentation, hemosiderin						5 (100%)
Capsule, mineralization, focal						4 (80%)
Lymphoid follicle, atrophy						4 (80%)
Red pulp, hematopoietic cell proliferation, diffuse				10 (100%)	10 (100%)	
Thymus	(10)	(1)	(2)	(10)	(10)	(3)
Atrophy					4 (40%)	2 (67%)
Congestion		1 (100%)	2 (100%)			

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Integumentary System						
Skin	(10)				(10)	(5)
Prepuce, inflammation, acute						1 (20%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(5)
Inflammation, chronic, focal					2 (20%)	
Metaplasia, osseous	1 (10%)					
Alveolar epithelium, hyperplasia, focal					1 (10%)	
Alveolus, infiltration cellular, focal, histiocyte	3 (30%)					1 (20%)
Nose	(10)				(10)	(5)
Respiratory epithelium, inflammation, chronic	1 (10%)					
Respiratory epithelium, metaplasia, squamous	1 (10%)					
Special Senses System						
Harderian gland				(1)		
Hemorrhage, acute				1 (100%)		
Urinary System						
Kidney	(10)	(4)	(3)	(10)	(10)	(5)
Cyst		1 (25%)				
Cortex, mineralization	1 (10%)					4 (80%)
Renal tubule, dilatation						1 (20%)
Renal tubule, regeneration	10 (100%)	4 (100%)	3 (100%)	9 (90%)	6 (60%)	
Urinary bladder	(10)			(1)	(10)	(5)
Calculus micro observation only	1 (10%)			1 (100%)	1 (10%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Early Deaths						
Moribund Sacrifice						7
Survivors						
Terminal Sacrifice	10	10	10	10	10	
Aborted						3
Animals Examined Microscopically	10	10	10	10	10	7
Alimentary System						
Intestine large, cecum	(10)				(10)	(7)
Parasite metazoan					1 (10%)	
Intestine large, colon	(10)				(10)	(7)
Parasite metazoan					1 (10%)	
Parasite metazoan, chronic					1 (10%)	
Intestine large, rectum	(10)				(10)	(7)
Parasite metazoan	2 (20%)				2 (20%)	
Liver	(10)	(10)	(10)	(10)	(10)	(7)
Developmental malformation				1 (10%)		
Hematopoietic cell proliferation					9 (90%)	
Hepatodiaphragmatic nodule	2 (20%)		3 (30%)	3 (30%)	1 (10%)	
Bile duct, hyperplasia, focal			1 (10%)			
Hepatocyte, centrilobular, degeneration						6 (86%)
Kupffer cell, pigmentation					10 (100%)	7 (100%)
Pancreas	(10)				(10)	(7)
Acinus, atrophy, focal	1 (10%)					
Pharynx						(1)
Palate, ulcer						1 (100%)
Salivary glands	(10)				(10)	(7)
Atrophy						7 (100%)
Stomach, glandular	(10)	(10)	(9)	(10)	(10)	(7)
Ectopic tissue					1 (10%)	
Ulcer						2 (29%)
Cardiovascular System						
Heart	(10)				(10)	(7)
Cardiomyopathy	5 (50%)				2 (20%)	
Endocrine System						
None						
General Body System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Genital System						
Clitoral gland	(10)	(10)	(10)	(9)	(10)	(7)
Atrophy					1 (10%)	7 (100%)
Cyst	1 (10%)	2 (20%)				
Dilatation		1 (10%)				
Inflammation, chronic, focal	1 (10%)				1 (10%)	
Ovary	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy						7 (100%)
Cyst	1 (10%)		1 (10%)	1 (10%)		
Uterus	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy					9 (90%)	7 (100%)
Dilatation	2 (20%)					
Vagina	(10)	(10)	(10)	(10)	(10)	(7)
Epithelium, atrophy					1 (10%)	7 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy						7 (100%)
Proliferation, diffuse					10 (100%)	
Lymph node	(10)			(3)	(10)	(7)
Mediastinal, atrophy						1 (14%)
Pancreatic, hyperplasia, lymphoid	1 (10%)					
Lymph node, mandibular	(10)			(2)	(10)	(7)
Atrophy						5 (71%)
Congestion				1 (50%)		
Lymph node, mesenteric	(10)				(9)	(7)
Atrophy						7 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(7)
Pigmentation, hemosiderin						7 (100%)
Capsule, mineralization, focal						5 (71%)
Lymphoid follicle, atrophy						6 (86%)
Red pulp, hematopoietic cell proliferation, diffuse					10 (100%)	
Thymus	(10)			(10)	(10)	(6)
Atrophy					10 (100%)	6 (100%)
Integumentary System						
Skin	(10)	(1)		(1)	(10)	(7)
Foot, developmental malformation		1 (100%)				
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Respiratory System						
Lung	(10)		(1)		(10)	(7)
Hemorrhage, acute, focal			1 (100%)		2 (20%)	
Inflammation, chronic, focal	4 (40%)				1 (10%)	
Alveolus, infiltration cellular, focal, histiocyte	2 (20%)				3 (30%)	2 (29%)
Special Senses System						
Eye	(1)					
Lens, cataract	1 (100%)					
Urinary System						
Kidney	(10)	(1)			(10)	(7)
Cyst, multiple		1 (100%)				
Developmental malformation					1 (10%)	
Inflammation, chronic, focal	2 (20%)				1 (10%)	
Cortex, mineralization	10 (100%)				10 (100%)	7 (100%)
Renal tubule, necrosis, focal						1 (14%)
Renal tubule, regeneration	1 (10%)				4 (40%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration		4 (40%)	8 (80%)	7 (70%)	10 (100%)	10 (100%)
Degeneration				8 (80%)	8 (80%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)	2 (20%)	3 (30%)	3 (30%)	1 (10%)	2 (20%)
Pigmentation						7 (70%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	6 (60%)					10 (100%)
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
General Body System						
None						
Genital System						
Seminal vesicle	(10)			(1)		(10)
Atrophy				1 (100%)		
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				2 (20%)	2 (20%)	8 (80%)
Lymph node	(10)			(1)	(2)	(10)
Mediastinal, angiectasis				1 (100%)		
Pancreatic, infiltration cellular, histiocyte					1 (50%)	
Lymph node, mandibular	(10)			(1)	(1)	(10)
Angiectasis				1 (100%)	1 (100%)	
Pigmentation						1 (10%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					2 (20%)	2 (20%)
Pigmentation			2 (20%)	10 (100%)	8 (80%)	10 (100%)

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Hemorrhage	1 (10%)					2 (20%)
Infiltration cellular, histiocyte	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cortex, mineralization	1 (10%)			2 (20%)	4 (40%)	1 (10%)
Renal tubule, regeneration	6 (60%)	6 (60%)	5 (50%)	7 (70%)	9 (90%)	5 (50%)
Urinary bladder	(10)	(3)	(1)	(1)	(1)	(10)
Calculus gross observation	1 (10%)	3 (100%)	1 (100%)		1 (100%)	
Calculus micro observation only	1 (10%)	3 (100%)	1 (100%)		1 (100%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration		5 (50%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Degeneration				10 (100%)	10 (100%)	10 (100%)
Hepatodiaphragmatic nodule	2 (20%)			1 (10%)	1 (10%)	
Pigmentation			2 (20%)	10 (100%)	10 (100%)	10 (100%)
Cardiovascular System						
Heart	(10)					(9)
Cardiomyopathy	1 (10%)					
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst						1 (10%)
General Body System						
None						
Genital System						
Ovary	(10)	(10)	(9)	(10)	(10)	(10)
Cyst			4 (44%)			1 (10%)
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				1 (10%)	9 (90%)	8 (80%)
Dilatation		2 (20%)				

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Hemapoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia					4 (40%)	3 (30%)
Lymph node	(10)		(1)			(10)
Pancreatic, infiltration cellular, histiocyte			1 (100%)			1 (10%)
Lymph node, mandibular	(10)					(10)
Angiectasis						1 (10%)
Infiltration cellular, histiocyte						1 (10%)
Lymph node, mesenteric	(10)					(8)
Infiltration cellular, histiocyte						1 (13%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion					1 (10%)	
Hematopoietic cell proliferation					6 (60%)	10 (100%)
Pigmentation			1 (10%)	9 (90%)	10 (100%)	9 (90%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(9)					(10)
Hemorrhage	1 (11%)					
Infiltration cellular, histiocyte						3 (30%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cortex, mineralization	10 (100%)	8 (80%)	8 (80%)	4 (40%)	7 (70%)	8 (80%)
Renal tubule, regeneration	3 (30%)	4 (40%)	8 (80%)	5 (50%)	8 (80%)	5 (50%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

Appendix B

Summary of Nonneoplastic Lesions in Mice

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(2)	(1)	(1)	(3)	(10)
Developmental malformation, focal						1 (10%)
Hematopoietic cell proliferation, focal	3 (30%)	1 (50%)				
Centrilobular, fatty change	1 (10%)		1 (100%)	1 (100%)	1 (33%)	
Tongue					(1)	
Mucosa, epithelium, hyperkeratosis, focal					1 (100%)	
Cardiovascular System						
None						
Endocrine System						
Parathyroid gland	(9)					(7)
Unilateral, cyst	1 (11%)					
General Body System						
None						
Genital System						
Preputial gland	(10)		(1)			(10)
Cyst			1 (100%)			
Testes	(10)	(9)	(10)	(10)	(10)	(10)
Seminiferous tubule, atrophy			3 (30%)	10 (100%)	10 (100%)	10 (100%)
Hemapoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation			10 (100%)	9 (90%)	9 (90%)	10 (100%)
Thymus	(9)			(10)	(10)	(10)
Depletion lymphoid					6 (60%)	9 (90%)
Integumentary System						
None						

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(2)	(2)		(10)
Congestion, focal			1 (50%)			
Hemorrhage, focal	4 (40%)	1 (100%)	1 (50%)	1 (50%)		2 (20%)
Special Senses System						
None						
Urinary System						
Kidney	(10)		(1)			(10)
Mineralization, focal	1 (10%)					
Interstitium, inflammation, focal, subacute						1 (10%)
Urinary bladder	(10)			(3)		(10)
Calculus gross observation				3 (100%)		
Lumen, calculus micro observation only				3 (100%)		

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)				(1)	(10)
Hematopoietic cell proliferation, focal	4 (40%)					
Centrilobular, fatty change	1 (10%)					
Parenchyma, ectopic tissue					1 (100%)	
Salivary glands	(10)					(10)
Inflammation, focal, subacute	2 (20%)					
Cardiovascular System						
None						
Endocrine System						
Adrenal gland, cortex	(10)	(10)	(9)	(10)	(10)	(10)
Zona reticularis, hypertrophy		10 (100%)	9 (100%)	10 (100%)	10 (100%)	10 (100%)
Parathyroid gland	(6)					(2)
Unilateral, cyst	1 (17%)					
General Body System						
None						
Genital System						
Ovary	(10)		(1)		(10)	(8)
Atrophy						5 (63%)
Perovarian tissue, inflammation, chronic, focal			1 (100%)			
Uterus	(10)	(10)	(10)	(10)	(10)	(8)
Endometrium, atrophy						1 (13%)
Lumen, dilatation	1 (10%)					
Hemapoietic System						
Lymph node, mandibular	(10)					(9)
Hyperplasia, lymphoid	1 (10%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation		5 (50%)	10 (100%)	8 (80%)	9 (90%)	10 (100%)
Thymus	(9)				(10)	(10)
Depletion lymphoid						4 (40%)

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(1)	(1)	(2)	(10)
Congestion				1 (100%)		
Hemorrhage, focal	2 (20%)				2 (100%)	3 (30%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, acute, focal	1 (10%)					
Mesentery				(1)		
Hemorrhage, focal				1 (100%)		
Fat, necrosis, focal				1 (100%)		
Pancreas	(10)			(1)		(10)
Duct, cyst, focal				1 (100%)		
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Aspermia						10 (100%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Seminiferous tubule, atrophy						10 (100%)
Hemapoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation						10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)				(3)	(10)
Bronchiole, inflammation, acute						1 (10%)
Interstitium, inflammation, acute, multifocal					3 (100%)	
Nose	(10)					(10)
Exudate						1 (10%)
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)				(1)	(10)
Calculus gross observation	1 (10%)				1 (100%)	
Calculus micro observation only	1 (10%)				1 (100%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, acute, focal	2 (20%)					
Centrilobular, hypertrophy		1 (10%)				
Cardiovascular System						
None						
Endocrine System						
Adrenal gland, cortex	(10)	(10)	(10)	(10)	(10)	(10)
Zona reticularis, hypertrophy			1 (10%)	8 (80%)	10 (100%)	9 (90%)
General Body System						
None						
Genital System						
Ovary	(10)		(10)	(10)	(10)	(10)
Bilateral, interstitium, atrophy						2 (20%)
Hematopoietic System						
Lymph node, mandibular	(10)		(1)			(10)
Hyperplasia, lymphoid			1 (100%)			
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid					2 (20%)	
Red pulp, hematopoietic cell proliferation				1 (10%)	9 (90%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)		(1)	(10)
Hemorrhage, focal	2 (20%)		1 (100%)		1 (100%)	2 (20%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	1		3	7	10
Alimentary System						
None						
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Hyperplasia	1 (10%)					
Hematopoietic System						
Lymph node, mandibular	(10)				(1)	(10)
Congestion	1 (10%)					1 (10%)
Lymph node, mesenteric	(9)					(10)
Hyperplasia						1 (10%)
Spleen	(10)				(1)	(10)
Developmental malformation					1 (100%)	
Integumentary System						
Skin	(10)					(10)
Sebaceous gland, hyperplasia	1 (10%)					
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F, Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)			(1)		(10)
Hemorrhage, focal	1 (10%)			1 (100%)		
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)					(10)
Calculus micro observation only	1 (10%)					2 (20%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F, Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals Initially In Study	10	10	10	10	10	10
Survivors						
Terminal Sacrifice	10	10	10	10	10	10
Animals Examined Microscopically	10	2		10	10	10
Alimentary System						
Liver	(10)	(1)		(1)		(10)
Inflammation, focal, subacute	2 (20%)					2 (20%)
Salivary glands	(10)					(10)
Parotid gland, inflammation, focal, subacute						1 (10%)
Stomach, forestomach	(10)					(10)
Hyperplasia, focal						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Parathyroid gland	(8)					(8)
Unilateral, cyst	1 (13%)					
General Body System						
None						
Genital System						
Uterus	(10)			(2)	(1)	(10)
Endometrium, hyperplasia				1 (50%)	1 (100%)	
Vagina	(10)			(10)	(10)	(10)
Developmental malformation						1 (10%)
Hemapoietic System						
Spleen	(10)	(1)				(10)
Hyperplasia, lymphoid		1 (100%)				
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)				(1)	(10)
Hemorrhage, focal					1 (100%)	3 (30%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX C

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	316 ± 7	295 ± 7	260 ± 5**	214 ± 5**	136 ± 20**[3]	–[4]
2-Ethoxyethanol	315 ± 5	309 ± 4	296 ± 4**	295 ± 8*	236 ± 5**	–
2-Butoxyethanol	308 ± 6	315 ± 4	309 ± 4	298 ± 3	280 ± 5**	264 ± 5**
Heart						
2-Methoxyethanol						
Absolute	1.084 ± 0.075	1.299 ± 0.105	1.120 ± 0.077	0.925 ± 0.045	0.705 ± 0.127[3]	–
Relative	3.42 ± 0.19	4.40 ± 0.32*	4.29 ± 0.23*	4.33 ± 0.21**	5.15 ± 0.18**[3]	–
2-Ethoxyethanol						
Absolute	1.240 ± 0.080	1.323 ± 0.053	1.235 ± 0.050	1.138 ± 0.054	1.071 ± 0.037	–
Relative	3.93 ± 0.22	4.30 ± 0.20	4.18 ± 0.16	3.86 ± 0.15	4.55 ± 0.16	–
2-Butoxyethanol						
Absolute	1.125 ± 0.050	1.162 ± 0.036	1.070 ± 0.026	1.148 ± 0.033	1.100 ± 0.033	1.061 ± 0.042
Relative	3.66 ± 0.19	3.69 ± 0.10	3.47 ± 0.06	3.85 ± 0.11	3.94 ± 0.13	4.03 ± 0.16
Right kidney						
2-Methoxyethanol						
Absolute	1.105 ± 0.034	1.051 ± 0.040	0.959 ± 0.023**	0.780 ± 0.027**	0.630 ± 0.060**[3]	–
Relative	3.50 ± 0.05	3.56 ± 0.07	3.70 ± 0.08	3.66 ± 0.13*	4.66 ± 0.24**[3]	–
2-Ethoxyethanol						
Absolute	1.079 ± 0.032	1.084 ± 0.028	1.043 ± 0.021	1.031 ± 0.028	0.901 ± 0.023**	–
Relative	3.42 ± 0.08	3.51 ± 0.06	3.53 ± 0.06	3.50 ± 0.04	3.83 ± 0.07**	–
2-Butoxyethanol						
Absolute	1.101 ± 0.028	1.255 ± 0.031	1.210 ± 0.031	1.093 ± 0.023	1.076 ± 0.016	1.074 ± 0.034
Relative	3.57 ± 0.04	3.98 ± 0.06**	3.91 ± 0.06**	3.66 ± 0.06*	3.85 ± 0.04**	4.07 ± 0.09**
Liver						
2-Methoxyethanol						
Absolute	10.16 ± 0.38	8.94 ± 0.41	7.93 ± 0.19**	6.87 ± 0.18**	5.04 ± 0.80**[3]	–
Relative	32.10 ± 0.70	30.30 ± 0.92	30.60 ± 0.57	32.20 ± 0.64	36.90 ± 0.43[3]	–
2-Ethoxyethanol						
Absolute	10.15 ± 0.31	9.95 ± 0.32	9.27 ± 0.19*	9.39 ± 0.30	6.51 ± 0.13**	–
Relative	32.20 ± 0.72	32.20 ± 0.93	31.40 ± 0.57	31.90 ± 0.59	27.60 ± 0.32**	–
2-Butoxyethanol						
Absolute	10.37 ± 0.35	10.93 ± 0.26	10.68 ± 0.23	10.35 ± 0.16	10.02 ± 0.25	9.71 ± 0.31
Relative	33.60 ± 0.56	34.70 ± 0.41	34.60 ± 0.69	34.70 ± 0.44	35.80 ± 0.45**	36.80 ± 0.86**
Lung						
2-Methoxyethanol						
Absolute	1.728 ± 0.024	1.852 ± 0.064	1.511 ± 0.066*	1.404 ± 0.076**	1.582 ± 0.194[3]	–
Relative	5.49 ± 0.14	6.28 ± 0.11**	5.81 ± 0.20*	6.53 ± 0.23**	11.66 ± 0.28**[3]	–
2-Ethoxyethanol						
Absolute	1.626 ± 0.094	1.679 ± 0.077	1.746 ± 0.100	1.543 ± 0.055[5]	1.374 ± 0.041*	–
Relative	5.15 ± 0.25	5.45 ± 0.26	5.91 ± 0.31	5.28 ± 0.13[5]	5.85 ± 0.19*	–
2-Butoxyethanol						
Absolute	1.710 ± 0.109	1.819 ± 0.125	1.585 ± 0.064	1.925 ± 0.195	1.507 ± 0.057	1.395 ± 0.022**
Relative	5.52 ± 0.28	5.78 ± 0.39	5.14 ± 0.21	6.47 ± 0.67	5.39 ± 0.16	5.31 ± 0.12

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Right testis						
2-Methoxyethanol						
Absolute	1.398 ± 0.048	1.411 ± 0.019	0.603 ± 0.044**	0.442 ± 0.032**	0.254 ± 0.010**[3]	–
Relative	4.44 ± 0.15	4.81 ± 0.09	2.31 ± 0.14**	2.07 ± 0.15**	1.89 ± 0.20**[3]	–
2-Ethoxyethanol						
Absolute	1.394 ± 0.022	1.431 ± 0.023	1.443 ± 0.016	1.342 ± 0.025	0.618 ± 0.042**	–
Relative	4.43 ± 0.05	4.64 ± 0.05	4.89 ± 0.06	4.56 ± 0.09	2.62 ± 0.18*	–
2-Butoxyethanol						
Absolute	1.399 ± 0.036	1.424 ± 0.020	1.407 ± 0.023	1.425 ± 0.021	1.352 ± 0.010	1.396 ± 0.013
Relative	4.54 ± 0.07	4.52 ± 0.04	4.56 ± 0.06	4.78 ± 0.08*	4.85 ± 0.08**	5.31 ± 0.10**
Thymus						
2-Methoxyethanol						
Absolute	0.268 ± 0.026	0.198 ± 0.017*	0.160 ± 0.016**	0.096 ± 0.016**	0.072 ± 0.005**[4]	–
Relative	0.85 ± 0.080	0.67 ± 0.05	0.61 ± 0.06	0.45 ± 0.07**	0.53 ± 0.04[3]	–
2-Ethoxyethanol						
Absolute	0.299 ± 0.019	0.270 ± 0.021	0.213 ± 0.005**	0.258 ± 0.010**	0.154 ± 0.011**	–
Relative	0.95 ± 0.05	0.87 ± 0.06	0.72 ± 0.02**	0.87 ± 0.02*	0.65 ± 0.05**	–
2-Butoxyethanol						
Absolute	0.309 ± 0.012	0.294 ± 0.017	0.291 ± 0.013	0.327 ± 0.022	0.256 ± 0.013**	0.262 ± 0.017*
Relative	1.01 ± 0.04	0.93 ± 0.05	0.94 ± 0.04	1.10 ± 0.08	0.92 ± 0.04	0.99 ± 0.06

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to animals given 2-methoxyethanol or 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm. Doses administered to animals given 2-ethoxyethanol were: 0, 1250, 2500, 5000, 10,000, or 20,000 ppm.

[3] n=2.

[4] n=0.

[5] n=9.

* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	189 ± 4	189 ± 2	170 ± 3**	145 ± 3**	151 ± 2**[3]	–[4]
2-Ethoxyethanol	185 ± 3	183 ± 3	177 ± 1	173 ± 3**	149 ± 1**	–
2-Butoxyethanol	186 ± 4	186 ± 3	181 ± 2	172 ± 2**	160 ± 2**	145 ± 2**
Heart						
2-Methoxyethanol						
Absolute	0.740 ± 0.028	0.702 ± 0.013	0.703 ± 0.033	0.653 ± 0.023*	0.649 ± 0.028[3]	–
Relative	3.94 ± 0.19	3.71 ± 0.09	4.17 ± 0.24	4.50 ± 0.14*	4.31 ± 0.18[3]	–
2-Ethoxyethanol						
Absolute	0.688 ± 0.033	0.717 ± 0.036	0.671 ± 0.012	0.674 ± 0.029	0.616 ± 0.019	–
Relative	3.73 ± 0.17	3.90 ± 0.15	3.80 ± 0.06	3.88 ± 0.13	4.14 ± 0.12**	–
2-Butoxyethanol						
Absolute	0.704 ± 0.028	0.697 ± 0.022	0.716 ± 0.027	0.686 ± 0.022	0.613 ± 0.019*	0.573 ± 0.016**
Relative	3.81 ± 0.20	3.75 ± 0.11	3.95 ± 0.16	3.99 ± 0.12	3.84 ± 0.11	3.94 ± 0.08
Right kidney						
2-Methoxyethanol						
Absolute	0.644 ± 0.015	0.656 ± 0.027	0.595 ± 0.016	0.544 ± 0.023**	0.577 ± 0.017**[3]	–
Relative	3.41 ± 0.07	3.46 ± 0.12	3.51 ± 0.08	3.76 ± 0.18**	3.83 ± 0.09**[3]	–
2-Ethoxyethanol						
Absolute	0.678 ± 0.008	0.683 ± 0.020	0.669 ± 0.010	0.663 ± 0.005	0.636 ± 0.010**	–
Relative	3.68 ± 0.07	3.74 ± 0.16	3.79 ± 0.07	3.83 ± 0.05	4.28 ± 0.06**	–
2-Butoxyethanol						
Absolute	0.668 ± 0.015	0.762 ± 0.010	0.759 ± 0.026	0.662 ± 0.011	0.668 ± 0.012	0.645 ± 0.011
Relative	3.59 ± 0.07	4.10 ± 0.06**	4.18 ± 0.12**	3.84 ± 0.04**	4.19 ± 0.09**	4.45 ± 0.09**
Liver						
2-Methoxyethanol						
Absolute	5.70 ± 0.13	5.66 ± 0.14	4.79 ± 0.17**	4.44 ± 0.21**	4.79 ± 0.33**[3]	–
Relative	30.10 ± 0.59	29.90 ± 0.67	28.20 ± 0.88	30.70 ± 1.70	31.80 ± 2.18[3]	–
2-Ethoxyethanol						
Absolute	5.38 ± 0.09	5.24 ± 0.17	5.08 ± 0.10	5.00 ± 0.10*	5.05 ± 0.11*	–
Relative	29.20 ± 0.65	28.50 ± 0.65	28.80 ± 0.45	28.90 ± 0.45	33.90 ± 0.72**	–
2-Butoxyethanol						
Absolute	5.56 ± 0.15	6.04 ± 0.16	6.00 ± 0.12	5.36 ± 0.10	5.13 ± 0.10	4.99 ± 0.11*
Relative	29.90 ± 0.54	32.50 ± 0.81*	33.00 ± 0.48**	31.10 ± 0.61*	32.20 ± 0.66*	34.40 ± 0.71**
Lung						
2-Methoxyethanol						
Absolute	1.133 ± 0.028	1.281 ± 0.049	1.167 ± 0.023	1.060 ± 0.039	1.061 ± 0.106[3]	–
Relative	5.99 ± 0.16	6.76 ± 0.20**	6.90 ± 0.20**	7.30 ± 0.24**	7.04 ± 0.66**[3]	–
2-Ethoxyethanol						
Absolute	1.109 ± 0.038	1.059 ± 0.028	1.102 ± 0.028	1.109 ± 0.048	0.991 ± 0.029*	–
Relative	6.01 ± 0.20	5.78 ± 0.13	6.24 ± 0.14	6.42 ± 0.30	6.66 ± 0.18**	–
2-Butoxyethanol						
Absolute	1.134 ± 0.035	1.056 ± 0.036	1.173 ± 0.037	1.134 ± 0.047	1.113 ± 0.095	1.025 ± 0.053
Relative	6.13 ± 0.25	5.68 ± 0.20	6.46 ± 0.19	6.59 ± 0.26	6.98 ± 0.59	7.05 ± 0.31*

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Thymus						
2-Methoxyethanol						
Absolute	0.224 ± 0.010	0.180 ± 0.012*	0.125 ± 0.010**	0.084 ± 0.008**	0.099 ± 0.011**[3]	—
Relative	1.19 ± 0.06	0.95 ± 0.06**	0.74 ± 0.06**	0.57 ± 0.06**	0.66 ± 0.07**[3]	—
2-Ethoxyethanol						
Absolute	0.214 ± 0.013	0.210 ± 0.007	0.221 ± 0.013	0.186 ± 0.009	0.069 ± 0.006**	—
Relative	1.16 ± 0.07	1.15 ± 0.04	1.25 ± 0.08	1.07 ± 0.05	0.47 ± 0.04**	—
2-Butoxyethanol						
Absolute	0.233 ± 0.015	0.232 ± 0.013	0.237 ± 0.013	0.213 ± 0.005	0.242 ± 0.015	0.173 ± 0.012*
Relative	1.26 ± 0.08	1.25 ± 0.08	1.31 ± 0.08	1.24 ± 0.03	1.51 ± 0.09	1.19 ± 0.09

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to animals given 2-methoxyethanol or 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm. Doses administered to animals given 2-ethoxyethanol were: 0, 1250, 2500, 5000, 10,000, or 20,000 ppm.

[3] n=5.

[4] n=0.

* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	39.2 ± 0.8	39.6 ± 0.8	40.8 ± 0.8	37.8 ± 0.9	37.1 ± 0.8[a]	30.1 ± 1.0**
2-Ethoxyethanol	38.9 ± 0.8	40.9 ± 0.8	43.0 ± 1.1	40.5 ± 0.9	33.6 ± 0.9*	31.9 ± 0.7**
2-Butoxyethanol	40.2 ± 1.6	40.1 ± 0.7	40.2 ± 0.5	38.4 ± 0.9	39.1 ± 0.8	38.3 ± 0.8
Heart						
2-Methoxyethanol						
Absolute	0.165 ± 0.006	0.173 ± 0.004	0.168 ± 0.003	0.172 ± 0.005	0.172 ± 0.004[a]	0.165 ± 0.004
Relative	4.24 ± 0.21	4.40 ± 0.15	4.13 ± 0.12	4.55 ± 0.11	4.64 ± 0.08[a,b]	5.52 ± 0.19**
2-Ethoxyethanol						
Absolute	0.183 ± 0.007	0.192 ± 0.007	0.198 ± 0.006	0.196 ± 0.008	0.171 ± 0.007	0.171 ± 0.007
Relative	4.70 ± 0.15	4.70 ± 0.19	4.63 ± 0.16	4.84 ± 0.19	5.15 ± 0.28	5.36 ± 0.16*
2-Butoxyethanol						
Absolute	0.173 ± 0.005	0.190 ± 0.006	0.186 ± 0.007[a]	0.179 ± 0.011	0.182 ± 0.009	0.175 ± 0.008
Relative	4.36 ± 0.20	4.74 ± 0.12	4.66 ± 0.18[a]	4.64 ± 0.23	4.64 ± 0.18	4.56 ± 0.16
Right kidney						
2-Methoxyethanol						
Absolute	0.288 ± 0.012	0.288 ± 0.006	0.293 ± 0.011	0.290 ± 0.007	0.340 ± 0.012**[a,b]	0.310 ± 0.013*
Relative	7.36 ± 0.30	7.30 ± 0.12	7.20 ± 0.29	7.66 ± 0.09	9.17 ± 0.23**[a,b]	10.32 ± 0.30**
2-Ethoxyethanol						
Absolute	0.341 ± 0.010	0.379 ± 0.010	0.367 ± 0.014	0.332 ± 0.012	0.331 ± 0.011	0.343 ± 0.010
Relative	8.78 ± 0.27	9.27 ± 0.20	8.54 ± 0.23	8.22 ± 0.32	9.88 ± 0.34*	10.75 ± 0.22**
2-Butoxyethanol						
Absolute	0.319 ± 0.009	0.385 ± 0.012	0.377 ± 0.007[a]	0.306 ± 0.007	0.319 ± 0.010	0.307 ± 0.008
Relative	7.98 ± 0.16	9.59 ± 0.20	9.41 ± 0.13[a]	7.99 ± 0.17	8.15 ± 0.18	8.02 ± 0.12
Liver						
2-Methoxyethanol						
Absolute	1.46 ± 0.09	1.50 ± 0.05	1.72 ± 0.05*	1.72 ± 0.07*	1.81 ± 0.07**[a]	1.58 ± 0.08*
Relative	37.30 ± 2.00	37.80 ± 0.71	42.20 ± 1.29*	45.20 ± 0.95**	48.80 ± 1.19**[a]	52.20 ± 1.50**
2-Ethoxyethanol						
Absolute	1.85 ± 0.06	2.03 ± 0.06	2.24 ± 0.10	2.00 ± 0.08	1.56 ± 0.07	1.75 ± 0.06
Relative	47.70 ± 1.42	49.50 ± 0.69	51.90 ± 1.38*	49.40 ± 1.59	46.30 ± 1.53	54.90 ± 1.23**
2-Butoxyethanol						
Absolute	1.56 ± 0.11	1.77 ± 0.04*	1.79 ± 0.03**[a]	1.48 ± 0.05	1.70 ± 0.07	1.58 ± 0.06
Relative	38.50 ± 1.45	44.20 ± 0.72**	44.70 ± 0.80**[a]	38.50 ± 0.85	43.30 ± 1.39	41.20 ± 1.11
Lung						
2-Methoxyethanol						
Absolute	0.246 ± 0.009	0.271 ± 0.022[a]	0.267 ± 0.013	0.267 ± 0.016	0.263 ± 0.013[a]	0.235 ± 0.006
Relative	6.26 ± 0.15	6.93 ± 0.68[a]	6.56 ± 0.33	7.07 ± 0.45	7.06 ± 0.22[a]	7.90 ± 0.38**
2-Ethoxyethanol						
Absolute	0.256 ± 0.007	0.289 ± 0.015	0.277 ± 0.012	0.269 ± 0.027	0.244 ± 0.013	0.251 ± 0.009
Relative	6.61 ± 0.23	7.08 ± 0.39	6.46 ± 0.29	6.63 ± 0.62	7.28 ± 0.35	7.90 ± 0.29**
2-Butoxyethanol						
Absolute	0.264 ± 0.016	0.315 ± 0.014	0.257 ± 0.011[a]	0.259 ± 0.018	0.235 ± 0.013	0.251 ± 0.018
Relative	6.62 ± 0.40	7.85 ± 0.28	6.39 ± 0.20[a]	6.76 ± 0.49	6.03 ± 0.36	6.54 ± 0.41

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Right testis						
2-Methoxyethanol						
Absolute	0.121 ± 0.002	0.120 ± 0.003	0.102 ± 0.003**	0.029 ± 0.002**	0.026 ± 0.001**[3]	0.023 ± 0.001**
Relative	3.11 ± 0.08	3.04 ± 0.06	2.51 ± 0.07**	0.77 ± 0.05**	0.69 ± 0.02**[3]	0.78 ± 0.06**
2-Ethoxyethanol						
Absolute	0.119 ± 0.002	0.124 ± 0.002	0.123 ± 0.005	0.119 ± 0.003	0.097 ± 0.004**	0.019 ± 0.002**
Relative	3.08 ± 0.08	3.05 ± 0.06	2.86 ± 0.11	2.95 ± 0.08	2.88 ± 0.11	0.59 ± 0.07**
2-Butoxyethanol						
Absolute	0.127 ± 0.002	0.126 ± 0.003	0.127 ± 0.004[3]	0.124 ± 0.002	0.122 ± 0.002	0.120 ± 0.002
Relative	3.19 ± 0.12	3.17 ± 0.11	3.17 ± 0.12[3]	3.23 ± 0.08	3.12 ± 0.07	3.15 ± 0.06
Thymus						
2-Methoxyethanol						
Absolute	0.046 ± 0.004	0.047 ± 0.004	0.047 ± 0.006	0.039 ± 0.002	0.036 ± 0.005**[4]	0.023 ± 0.003**
Relative	1.17 ± 0.08	1.18 ± 0.09	1.15 ± 0.14	1.04 ± 0.07	0.98 ± 0.12**[3]	0.76 ± 0.09**
2-Ethoxyethanol						
Absolute	0.055 ± 0.004	0.058 ± 0.005	0.057 ± 0.005	0.060 ± 0.004	0.041 ± 0.006	0.043 ± 0.004*
Relative	1.42 ± 0.11	1.40 ± 0.11	1.31 ± 0.09	1.47 ± 0.09	1.21 ± 0.16	1.33 ± 0.11
2-Butoxyethanol						
Absolute	0.052 ± 0.005	0.054 ± 0.004	0.050 ± 0.004[3]	0.050 ± 0.007	0.045 ± 0.004	0.041 ± 0.004
Relative	1.28 ± 0.09	1.35 ± 0.06	1.25 ± 0.10[3]	1.27 ± 0.16	1.16 ± 0.10	1.06 ± 0.08

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to mice given 2-methoxyethanol were: 0, 2000, 4000, 6000, 8000 or 10,000 ppm. Doses given to mice administered 2-ethoxyethanol were: 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. Doses administered to mice given 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000.

[3] n=9.

* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	29.7 ± 0.7	29.3 ± 0.7	29.6 ± 1.0	27.2 ± 1.2	26.0 ± 0.9**	23.9 ± 0.9**
2-Ethoxyethanol	31.3 ± 0.8	31.8 ± 1.2	33.2 ± 1.0	29.9 ± 1.5	27.8 ± 0.8*	24.8 ± 0.5**
2-Butoxyethanol	31.1 ± 0.7	31.8 ± 0.8	30.9 ± 1.5	28.0 ± 0.7*	28.4 ± 0.5*	27.8 ± 0.9**
Heart						
2-Methoxyethanol						
Absolute	0.123 ± 0.003	0.130 ± 0.006	0.144 ± 0.006*	0.127 ± 0.005	0.130 ± 0.004	0.127 ± 0.004
Relative	4.16 ± 0.18	4.44 ± 0.19	4.88 ± 0.11**	4.81 ± 0.35*	5.04 ± 0.19**	5.38 ± 0.15**
2-Ethoxyethanol						
Absolute	0.136 ± 0.007	0.138 ± 0.004	0.135 ± 0.004	0.129 ± 0.004	0.139 ± 0.003	0.134 ± 0.004
Relative	4.33 ± 0.13	4.43 ± 0.25	4.11 ± 0.21	4.38 ± 0.21	5.02 ± 0.13*	5.41 ± 0.14**
2-Butoxyethanol						
Absolute	0.132 ± 0.005	0.143 ± 0.004	0.141 ± 0.006	0.130 ± 0.004	0.130 ± 0.004	0.129 ± 0.006
Relative	4.27 ± 0.17	4.50 ± 0.18	4.67 ± 0.31	4.66 ± 0.12	4.60 ± 0.17	4.68 ± 0.22
Right kidney						
2-Methoxyethanol						
Absolute	0.185 ± 0.004	0.227 ± 0.005**	0.212 ± 0.010*	0.206 ± 0.010	0.201 ± 0.005	0.209 ± 0.008
Relative	6.26 ± 0.18	7.80 ± 0.27**	7.18 ± 0.33**	7.73 ± 0.57*	7.82 ± 0.34**	8.88 ± 0.39**
2-Ethoxyethanol						
Absolute	0.208 ± 0.005	0.236 ± 0.006*	0.207 ± 0.006	0.204 ± 0.010	0.206 ± 0.005	0.241 ± 0.004**
Relative	6.71 ± 0.27	7.49 ± 0.23*	6.25 ± 0.18	6.87 ± 0.20	7.45 ± 0.18*	9.73 ± 0.09**
2-Butoxyethanol						
Absolute	0.196 ± 0.004	0.244 ± 0.004**	0.245 ± 0.006**	0.209 ± 0.008	0.214 ± 0.004	0.227 ± 0.007*
Relative	6.33 ± 0.10	7.69 ± 0.14**	8.06 ± 0.29**	7.47 ± 0.19**	7.55 ± 0.18**	8.21 ± 0.26**
Liver						
2-Methoxyethanol						
Absolute	1.24 ± 0.05	1.38 ± 0.05	1.36 ± 0.06	1.26 ± 0.04	1.19 ± 0.05	1.18 ± 0.03
Relative	42.10 ± 2.17	47.00 ± 1.57	46.00 ± 1.46	47.60 ± 3.27	46.10 ± 2.59	49.90 ± 1.82**
2-Ethoxyethanol						
Absolute	1.22 ± 0.03	1.41 ± 0.05*	1.20 ± 0.05	1.23 ± 0.08	1.25 ± 0.04	1.22 ± 0.03
Relative	39.30 ± 1.25	44.60 ± 1.33**	36.30 ± 1.37	40.90 ± 0.85	45.00 ± 1.21**	49.10 ± 0.79**
2-Butoxyethanol						
Absolute	1.18 ± 0.04	1.36 ± 0.05	1.37 ± 0.05	1.16 ± 0.04	1.16 ± 0.04	1.16 ± 0.05
Relative	38.20 ± 1.25	42.70 ± 1.40	44.60 ± 1.12**	41.60 ± 0.92	41.00 ± 1.15	41.70 ± 1.15
Lung						
2-Methoxyethanol						
Absolute	0.241 ± 0.014	0.239 ± 0.006	0.242 ± 0.022	0.274 ± 0.015	0.251 ± 0.016	0.253 ± 0.021
Relative	8.11 ± 0.45	8.20 ± 0.32	8.18 ± 0.67	10.22 ± 0.61*	9.71 ± 0.59*	10.54 ± 0.70*
2-Ethoxyethanol						
Absolute	0.232 ± 0.008	0.240 ± 0.013	0.245 ± 0.015	0.248 ± 0.016	0.265 ± 0.020	0.209 ± 0.006
Relative	7.46 ± 0.31	7.65 ± 0.49	7.46 ± 0.53	8.44 ± 0.62	9.56 ± 0.67*	8.43 ± 0.16*
2-Butoxyethanol						
Absolute	0.263 ± 0.017	0.227 ± 0.009	0.235 ± 0.010	0.254 ± 0.017	0.259 ± 0.018	0.240 ± 0.016
Relative	8.51 ± 0.60	7.13 ± 0.22	7.68 ± 0.26	9.04 ± 0.56	9.16 ± 0.65	8.64 ± 0.50

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Thymus						
2-Methoxyethanol						
Absolute	0.048 ± 0.003	0.055 ± 0.005	0.049 ± 0.003	0.042 ± 0.002	0.037 ± 0.003*	0.026 ± 0.003**
Relative	1.63 ± 0.09	1.89 ± 0.19	1.67 ± 0.09	1.57 ± 0.10	1.46 ± 0.13	1.09 ± 0.14*
2-Ethoxyethanol						
Absolute	0.057 ± 0.003	0.055 ± 0.004	0.056 ± 0.004	0.056 ± 0.005	0.056 ± 0.003	0.043 ± 0.003**
Relative	1.84 ± 0.12	1.71 ± 0.12	1.69 ± 0.13	1.88 ± 0.12	2.04 ± 0.11	1.71 ± 0.12
2-Butoxyethanol						
Absolute	0.063 ± 0.005	0.062 ± 0.005	0.055 ± 0.003	0.051 ± 0.002*	0.051 ± 0.004*	0.054 ± 0.004
Relative	2.02 ± 0.14	1.94 ± 0.13	1.80 ± 0.08	1.82 ± 0.07	1.80 ± 0.12	1.91 ± 0.09

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to mice given 2-methoxyethanol were: 0, 2000, 4000, 6000, 8000 or 10,000 ppm. Doses given to mice administered 2-ethoxyethanol were: 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. Doses administered to mice given 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's test or Shirley's test.

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]			
	Dose 1	Dose 2	Dose 3	Dose 4
Day 60				
n	10	10	10	10
Necropsy body wt				
2-Methoxyethanol	294 ± 4	248 ± 6**	228 ± 7**	–[3]
2-Ethoxyethanol	306 ± 7	285 ± 6*	259 ± 5**	138 ± 21**[4]
2-Butoxyethanol	299 ± 5	282 ± 6	275 ± 4**	255 ± 4**
Right testis				
2-Methoxyethanol				
Absolute	1.412 ± 0.016	0.644 ± 0.028**	0.433 ± 0.015**	–
Relative	4.81 ± 0.07	2.59 ± 0.09**	1.90 ± 0.06**	–
2-Ethoxyethanol				
Absolute	1.368 ± 0.019	1.400 ± 0.016	0.609 ± 0.044**	0.361 ± 0.096**[4]
Relative	4.48 ± 0.09	4.93 ± 0.10	2.37 ± 0.19**	2.51 ± 0.27**[4]
2-Butoxyethanol				
Absolute	1.47 ± 0.02	1.38 ± 0.02**	1.35 ± 0.02**	1.34 ± 0.01**
Relative	4.91 ± 0.05	4.90 ± 0.08	4.91 ± 0.07	5.25 ± 0.07**
Epididymis				
2-Methoxyethanol				
Absolute	0.485 ± 0.017	0.281 ± 0.019**	0.237 ± 0.015**	–
Relative	1.65 ± 0.06	1.13 ± 0.07**	1.04 ± 0.05**	–
2-Ethoxyethanol				
Absolute	0.441 ± 0.012	0.420 ± 0.014	0.228 ± 0.012**	0.114 ± 0.018**[4]
Relative	1.44 ± 0.03	1.48 ± 0.06	0.88 ± 0.04**	0.83 ± 0.06**[4]
2-Butoxyethanol				
Absolute	0.472 ± 0.012	0.465 ± 0.016	0.450 ± 0.011	0.446 ± 0.014
Relative	1.58 ± 0.03	1.66 ± 0.07	1.64 ± 0.04	1.75 ± 0.04**
Day 90				
n	10	10	10	10
Necropsy body wt				
2-Methoxyethanol	339 ± 7	311 ± 7*	278 ± 5**	–
2-Ethoxyethanol	339 ± 8	339 ± 6	303 ± 3**	237 ± 37**[4]
2-Butoxyethanol	332 ± 6	331 ± 5	321 ± 11	329 ± 7
Right testis				
2-Methoxyethanol				
Absolute	1.432 ± 0.022	0.846 ± 0.051**	0.442 ± 0.015**	–
Relative	4.23 ± 0.07	2.74 ± 0.18**	1.59 ± 0.05**	–
2-Ethoxyethanol				
Absolute	1.460 ± 0.030	1.415 ± 0.021	0.652 ± 0.029**	0.395 ± 0.038**[4]
Relative	4.32 ± 0.05	4.19 ± 0.10	2.15 ± 0.10**	1.72 ± 0.10**[4]
2-Butoxyethanol				
Absolute	1.43 ± 0.02	1.48 ± 0.01	1.46 ± 0.03	1.40 ± 0.03
Relative	4.31 ± 0.07	4.48 ± 0.06	4.61 ± 0.20	4.26 ± 0.15

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats In the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose			
	Dose 1	Dose 2	Dose 3	Dose 4
Day 90 (continued)				
Epididymis				
2-Methoxyethanol				
Absolute	0.480 ± 0.015	0.308 ± 0.012**	0.261 ± 0.011**	–
Relative	1.41 ± 0.04	1.00 ± 0.05**	0.94 ± 0.04**	–
2-Ethoxyethanol				
Absolute	0.507 ± 0.018	0.497 ± 0.017	0.311 ± 0.015**	0.204 ± 0.014**[6]
Relative	1.49 ± 0.04	1.47 ± 0.05	1.03 ± 0.05**	0.91 ± 0.11**[6]
2-Butoxyethanol				
Absolute	0.520 ± 0.034	0.445 ± 0.020	0.475 ± 0.019	0.464 ± 0.017
Relative	1.57 ± 0.10	1.34 ± 0.05	1.50 ± 0.08	1.41 ± 0.06
Day 116				
n	10	10	10	5
Necropsy body wt				
2-Methoxyethanol	381 ± 8	343 ± 6**	324 ± 7**	–
2-Ethoxyethanol	384 ± 6	362 ± 8*	352 ± 6**[6]	272 ± 29**
Right testis				
2-Methoxyethanol				
Absolute	1.534 ± 0.024	0.914 ± 0.057**	0.478 ± 0.044**	–
Relative	4.04 ± 0.08	2.66 ± 0.14**	1.47 ± 0.12**	–
2-Ethoxyethanol				
Absolute	1.486 ± 0.022	1.362 ± 0.026**	0.678 ± 0.044**[6]	0.444 ± 0.023**
Relative	3.88 ± 0.07	3.77 ± 0.06	1.92 ± 0.12**[6]	1.72 ± 0.23**
Epididymis				
2-Methoxyethanol				
Absolute	0.544 ± 0.016	0.366 ± 0.025**	0.277 ± 0.016**	–
Relative	1.43 ± 0.05	1.06 ± 0.06**	0.86 ± 0.05**	–
2-Ethoxyethanol				
Absolute	0.533 ± 0.015	0.544 ± 0.021	0.319 ± 0.019**[6]	0.255 ± 0.024**
Relative	1.39 ± 0.04	1.51 ± 0.06	0.91 ± 0.05**[6]	0.95 ± 0.04**

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 1500, 3000, or 6000 ppm. Doses administered to rats given 2-ethoxyethanol were: 0, 5000, 10,000, or 20,000 ppm.

[3] Data not available due to 100% mortality in the 6000 ppm 2-methoxyethanol group.

[4] n=4.

[5] n=5.

[6] n=9.

* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

APPENDIX D

Hematology, Clinical Chemistry, and Urinalysis Results

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE						
Hematology						
n						
Week 1	10	10	10	10	9	9
Week 3	10	8	10	9	0	0
Week 13	8	9	10	10	2	0
Hematocrit (%)						
Week 1	46.1 ± 0.6	45.8 ± 0.5	46.2 ± 0.6	44.3 ± 0.4**	45.4 ± 0.3	43.6 ± 0.5**
Week 3	49.3 ± 0.6	45.6 ± 0.6**	46.2 ± 0.6**	41.6 ± 0.5**	–	–
Week 13	48.1 ± 0.4	46.9 ± 0.6	45.4 ± 0.7**	46.0 ± 0.8*	31.6 ± 7.0**	–
Hemoglobin (g/dL)						
Week 1	15.0 ± 0.1	14.7 ± 0.1	14.8 ± 0.2	14.3 ± 0.1**	14.4 ± 0.2**	13.9 ± 0.2**
Week 3	16.0 ± 0.2	14.9 ± 0.2**	14.9 ± 0.2**	13.8 ± 0.1**	–	–
Week 13	16.0 ± 0.2	15.5 ± 0.2	15.2 ± 0.2**	14.9 ± 0.2**	10.1 ± 1.9**	–
Erythrocytes (10^6/μL)						
Week 1	7.88 ± 0.12	7.88 ± 0.10	7.96 ± 0.12	7.60 ± 0.07	7.70 ± 0.11	7.44 ± 0.11*
Week 3	8.80 ± 0.10	8.32 ± 0.14**	8.47 ± 0.14**	7.61 ± 0.10**	–	–
Week 13	9.44 ± 0.11	9.40 ± 0.13	9.20 ± 0.13	9.08 ± 0.16	5.94 ± 1.24**	–
Reticulocytes (10^6/μL)						
Week 1	0.22 ± 0.03	0.27 ± 0.02[2]	0.21 ± 0.02	0.12 ± 0.02**	0.07 ± 0.01**	0.05 ± 0.01**
Week 3	0.18 ± 0.01	0.17 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	–	–
Week 13	0.12 ± 0.01	0.17 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.09 ± 0.03	–
Nucleated erythrocytes (10^3/μL)						
Week 1	0.02 ± 0.01[2]	0.07 ± 0.03[2]	0.02 ± 0.01[2]	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00[3]	–	–
Week 13	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	–
Mean cell volume (fL)						
Week 1	58.6 ± 0.3	58.0 ± 0.3	58.0 ± 0.4	58.3 ± 0.4	59.1 ± 0.4	58.6 ± 0.2
Week 3	56.1 ± 0.3	54.9 ± 0.4**	54.6 ± 0.2**	54.8 ± 0.2**	–	–
Week 13	50.9 ± 0.3	49.8 ± 0.5	49.1 ± 0.2**	50.8 ± 0.2	53.0 ± 1.0	–
Mean cell hemoglobin (pg)						
Week 1	19.0 ± 0.2	18.6 ± 0.1	18.6 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.8 ± 0.1
Week 3	18.2 ± 0.1	18.0 ± 0.2	17.6 ± 0.1**	18.1 ± 0.1	–	–
Week 13	16.9 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.2	17.0 ± 0.4	–
Mean cell hemoglobin concentration (g/dL)						
Week 1	32.4 ± 0.3	32.1 ± 0.2	32.0 ± 0.2	32.2 ± 0.2	31.7 ± 0.2*	32.0 ± 0.1
Week 3	32.5 ± 0.2	32.8 ± 0.4	32.3 ± 0.1	33.1 ± 0.2**	–	–
Week 13	33.2 ± 0.3	33.1 ± 0.4	33.5 ± 0.3	32.4 ± 0.4	32.1 ± 1.2	–
Platelets (10^3/μL)						
Week 1	937.5 ± 31.3	864.8 ± 12.1**	791.8 ± 13.0**	492.5 ± 18.6**	338.1 ± 21.0**	276.2 ± 20.8**
Week 3	797.7 ± 13.3	730.1 ± 16.5**	568.7 ± 11.8**	267.7 ± 7.9**	–	–
Week 13	582.4 ± 12.1	612.8 ± 18.0	490.9 ± 13.5**	401.9 ± 33.8**	265.5 ± 53.5**	–
Leukocytes (10^3/μL)						
Week 1	7.87 ± 0.51	7.45 ± 0.45	7.05 ± 0.37	4.94 ± 0.29**	3.37 ± 0.34**	2.92 ± 0.22**
Week 3	8.49 ± 0.40	7.68 ± 0.35	6.81 ± 0.46**	4.81 ± 0.19**	–	–
Week 13	7.49 ± 0.63	8.51 ± 0.73	6.47 ± 0.61	6.18 ± 0.54	1.80 ± 0.30*	–

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	0.96 ± 0.12[a]	0.75 ± 0.08[a]	1.04 ± 0.13[a]	0.77 ± 0.12	0.51 ± 0.13*	0.39 ± 0.05**
Week 3	1.02 ± 0.06	1.07 ± 0.08	0.68 ± 0.08**	0.63 ± 0.11**	—	—
Week 13	1.26 ± 0.20	1.19 ± 0.14	1.06 ± 0.16	0.79 ± 0.07*	0.25 ± 0.06**	—
Lymphocytes (10^3/μL)						
Week 1	6.97 ± 0.56[a]	6.80 ± 0.38[a]	5.71 ± 0.32[a]	4.09 ± 0.18**	2.78 ± 0.24**	2.50 ± 0.21**
Week 3	7.36 ± 0.43	6.47 ± 0.31	6.03 ± 0.41*	4.14 ± 0.20**	—	—
Week 13	6.09 ± 0.45	7.17 ± 0.61	5.32 ± 0.51	5.19 ± 0.45	1.51 ± 0.25*	—
Monocytes (10^3/μL)						
Week 1	0.03 ± 0.02[a]	0.11 ± 0.03[a]	0.09 ± 0.03[a]	0.05 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
Week 3	0.08 ± 0.03	0.11 ± 0.03	0.09 ± 0.02	0.04 ± 0.01	—	—
Week 13	0.08 ± 0.03	0.10 ± 0.03	0.05 ± 0.03	0.15 ± 0.04	0.02 ± 0.02	—
Eosinophils (10^3/μL)						
Week 1	0.02 ± 0.01[a]	0.01 ± 0.01[a]	0.05 ± 0.03[a]	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
Week 3	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	—	—
Week 13	0.05 ± 0.03	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.01 ± 0.01	—
Methemoglobin (g/dL)						
Week 1	0.09 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.02	0.07 ± 0.02	0.10 ± 0.02
Week 3	0.11 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.02	—	—
Week 13	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.06	—
Total bone marrow cellularity (10^6/femur)						
Week 1	70.6 ± 2.7	—[d]	66.6 ± 3.2	53.5 ± 2.9**	32.7 ± 2.0**[e]	25.5 ± 1.2**[e]
Week 3	66.1 ± 2.9	82.2 ± 3.6[b]	75.1 ± 3.9	53.2 ± 2.4	—	—
Week 13	66.0 ± 2.9[b]	71.1 ± 3.0[b]	58.4 ± 2.1	57.0 ± 2.2*	31.4 ± 12.2**	—
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	9	0	0
Week 13	10	10	10	10	2	0
Blood urea nitrogen (mg/dL)						
Week 1	18.6 ± 0.6	19.7 ± 1.0	16.9 ± 0.6	16.0 ± 0.6	18.2 ± 0.7	17.4 ± 0.9
Week 3	20.4 ± 0.4	18.4 ± 0.6	20.5 ± 0.8	21.1 ± 0.9	—	—
Week 13	16.7 ± 1.1	17.9 ± 0.9	20.0 ± 1.6	16.9 ± 0.7	34.5 ± 14.5	—
Creatinine (mg/dL)						
Week 1	0.45 ± 0.02	0.41 ± 0.01	0.41 ± 0.01*	0.38 ± 0.01**	0.39 ± 0.02**	0.36 ± 0.02**
Week 3	0.59 ± 0.02	0.60 ± 0.02	0.56 ± 0.02	0.50 ± 0.00**	—	—
Week 13	0.55 ± 0.03	0.50 ± 0.02	0.49 ± 0.01	0.49 ± 0.02	0.35 ± 0.05**	—
Total protein (g/dL)						
Week 1	6.1 ± 0.0	5.9 ± 0.1*	5.8 ± 0.1**	5.5 ± 0.0**	5.6 ± 0.1**	5.4 ± 0.1**
Week 3	6.3 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	5.6 ± 0.0**	—	—
Week 13	6.6 ± 0.1	6.4 ± 0.1	6.2 ± 0.1**	6.0 ± 0.1**	5.0 ± 0.1**	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1*	3.2 ± 0.1*	3.0 ± 0.1**
Week 3	3.7 ± 0.0	3.5 ± 0.1*	3.6 ± 0.0*	3.3 ± 0.1**	—	—
Week 13	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.5 ± 0.0	2.8 ± 0.3**	—
Alkaline phosphatase (IU/L)						
Week 1	442 ± 8	401 ± 10**	364 ± 10**	321 ± 14**	317 ± 18**	308 ± 8**
Week 3	271 ± 5	281 ± 10	238 ± 8*	137 ± 5**	—	—
Week 13	138 ± 2	131 ± 2	135 ± 5	152 ± 8	89 ± 10	—
Alanine aminotransferase (IU/L)						
Week 1	34 ± 2	36 ± 2	32 ± 1	34 ± 1	37 ± 2	31 ± 1
Week 3	35 ± 1	32 ± 1	33 ± 1	27 ± 1**	—	—
Week 13	34 ± 1	33 ± 1	33 ± 1	36 ± 1	31 ± 0	—
Creatine kinase (IU/L)						
Week 1	412 ± 39	415 ± 44	436 ± 52	425 ± 60[a]	395 ± 36	304 ± 29
Week 3	153 ± 25[a]	212 ± 27	187 ± 23	93 ± 12	—	—
Week 13	79 ± 10	143 ± 27	133 ± 18	87 ± 10	89 ± 24	—
Bile acids (µmol/L)						
Week 1	10.10 ± 0.85	13.70 ± 1.12*	15.30 ± 3.84	14.50 ± 1.93*	25.30 ± 5.14**	16.80 ± 0.76**
Week 3	9.30 ± 1.56	11.44 ± 1.36[a]	23.50 ± 4.66**	33.78 ± 7.85**	—	—
Week 13	17.20 ± 4.57	19.40 ± 3.34	11.70 ± 1.41	18.50 ± 3.79	16.00 ± 3.00	—
Urinalysis						
n	10	10	10	10	2	0
Urine volume (mL/16 hr)						
Week 13	5.6 ± 0.3	4.8 ± 0.4[a]	3.9 ± 0.2**	3.8 ± 0.3**	6.8 ± 2.8	—
Specific gravity						
Week 13	1.042 ± 0.002	1.045 ± 0.004	1.064 ± 0.003**	1.063 ± 0.003**	1.046 ± 0.004	—
Urine pH						
Week 13	7.40 ± 0.22	6.75 ± 0.08	6.65 ± 0.08*	6.95 ± 0.19	7.00 ± 0.00	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE						
Hematology						
n						
Week 1	10	10	10	10	10	10
Week 3	7	9	8	5	0	0
Week 13	9	9	8	10	5	0
Hematocrit (%)						
Week 1	46.8 ± 0.4	45.6 ± 0.7	44.8 ± 0.6*	43.2 ± 0.5**	43.7 ± 0.6**	43.4 ± 0.7**
Week 3	48.6 ± 0.6	48.4 ± 0.5	47.4 ± 0.6	43.1 ± 1.2**	—	—
Week 13	44.5 ± 0.4	43.8 ± 0.4	42.2 ± 0.8*	41.5 ± 0.5**	40.7 ± 0.9**	—
Hemoglobin (g/dL)						
Week 1	15.8 ± 0.1	15.4 ± 0.2	15.1 ± 0.2*	14.5 ± 0.1**	14.8 ± 0.2**	14.9 ± 0.2**
Week 3	16.0 ± 0.1	15.8 ± 0.1	15.8 ± 0.2	14.3 ± 0.2**	—	—
Week 13	15.2 ± 0.1	14.8 ± 0.1*	14.5 ± 0.2**	13.7 ± 0.1**	13.6 ± 0.3**	—
Erythrocytes (10^6/μL)						
Week 1	8.14 ± 0.09	7.94 ± 0.12	7.86 ± 0.12	7.43 ± 0.10**	7.66 ± 0.15**	7.57 ± 0.12**
Week 3	8.73 ± 0.11	8.80 ± 0.12	8.85 ± 0.14	8.09 ± 0.22	—	—
Week 13	8.34 ± 0.09	8.30 ± 0.09	8.24 ± 0.13	8.13 ± 0.12	7.91 ± 0.18	—
Reticulocytes (10^6/μL)						
Week 1	0.22 ± 0.02	0.15 ± 0.02*	0.09 ± 0.00**	0.05 ± 0.01**	0.03 ± 0.00**	0.03 ± 0.00**
Week 3	0.16 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.18 ± 0.02	—	—
Week 13	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.09 ± 0.01	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.00 ± 0.00	0.00 ± 0.00[b]	0.01 ± 0.01	0.00 ± 0.00	—	—
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	—
Mean cell volume (fL)						
Week 1	57.5 ± 0.2	57.5 ± 0.3	57.2 ± 0.4	58.2 ± 0.3	57.1 ± 0.4	57.4 ± 0.3
Week 3	55.7 ± 0.3	55.1 ± 0.2	53.6 ± 0.3**	53.2 ± 0.2**	—	—
Week 13	53.3 ± 0.3	52.8 ± 0.2	51.3 ± 0.3**	50.9 ± 0.3**	51.6 ± 0.2**	—
Mean cell hemoglobin (pg)						
Week 1	19.5 ± 0.2	19.4 ± 0.2	19.3 ± 0.1	19.5 ± 0.2	19.3 ± 0.2	19.7 ± 0.1
Week 3	18.3 ± 0.2	18.0 ± 0.2	18.0 ± 0.2	17.8 ± 0.2	—	—
Week 13	18.3 ± 0.2	17.9 ± 0.1	17.6 ± 0.1**	16.9 ± 0.2**	17.3 ± 0.1**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.9 ± 0.3	33.8 ± 0.3	33.8 ± 0.3	33.5 ± 0.3	33.9 ± 0.3	34.3 ± 0.2
Week 3	32.9 ± 0.3	32.7 ± 0.3	33.2 ± 0.2	33.3 ± 0.4	—	—
Week 13	34.2 ± 0.3	33.8 ± 0.3	34.3 ± 0.3	33.1 ± 0.3*	33.5 ± 0.2	—
Platelets (10^3/μL)						
Week 1	852.8 ± 19.7	775.3 ± 14.6*	539.0 ± 12.9**	261.6 ± 10.6**	180.1 ± 22.3**	159.9 ± 21.7**
Week 3	861.4 ± 20.1	658.0 ± 11.3**	531.1 ± 13.7**	349.6 ± 20.7**	—	—
Week 13	658.9 ± 24.3	650.6 ± 12.0	534.9 ± 25.4**	400.7 ± 27.2**	376.0 ± 32.0**	—
Leukocytes (10^3/μL)						
Week 1	9.24 ± 0.36	7.35 ± 0.35**	5.80 ± 0.39**	4.49 ± 0.23**	3.51 ± 0.37**	3.45 ± 0.30**
Week 3	7.87 ± 0.56	7.48 ± 0.39	8.24 ± 0.61	5.36 ± 0.52*	—	—
Week 13	7.14 ± 0.23	6.76 ± 0.18	5.74 ± 0.26**	4.16 ± 0.45**	4.62 ± 0.50**	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	1.07 ± 0.19	0.69 ± 0.07	0.75 ± 0.08	0.54 ± 0.06**	0.42 ± 0.08**	0.43 ± 0.10**
Week 3	0.97 ± 0.10	0.85 ± 0.14	0.91 ± 0.11	0.79 ± 0.12	–	–
Week 13	0.94 ± 0.14	0.97 ± 0.12	0.75 ± 0.06	0.48 ± 0.08**	0.53 ± 0.15*	–
Lymphocytes (10^3/μL)						
Week 1	8.02 ± 0.31	6.55 ± 0.34**	4.96 ± 0.36**	3.91 ± 0.19**	3.03 ± 0.33**	2.95 ± 0.24**
Week 3	6.79 ± 0.47	6.53 ± 0.40	7.22 ± 0.58	4.44 ± 0.49	–	–
Week 13	6.08 ± 0.34	5.63 ± 0.12	4.80 ± 0.24**	3.56 ± 0.43**	4.00 ± 0.46**	–
Monocytes (10^3/μL)						
Week 1	0.12 ± 0.03	0.09 ± 0.03	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.02
Week 3	0.08 ± 0.03	0.09 ± 0.03	0.08 ± 0.03	0.10 ± 0.06	–	–
Week 13	0.06 ± 0.03	0.10 ± 0.03	0.08 ± 0.03	0.07 ± 0.02	0.02 ± 0.01	–
Eosinophils (10^3/μL)						
Week 1	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	–	–
Week 13	0.06 ± 0.02	0.06 ± 0.02	0.11 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	–
Methemoglobin (g/dL)						
Week 1	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.03
Week 3	0.15 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	–	–
Week 13	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	–
Total bone marrow cellularity (10^6/femur)						
Week 1	55.2 ± 2.4	–[4]	43.6 ± 2.0**	25.9 ± 1.1**	21.5 ± 1.4**	19.9 ± 1.3**
Week 3	46.2 ± 1.4[5]	40.6 ± 1.8**[5]	34.7 ± 1.5**[5]	30.2 ± 2.7**	–	–
Week 13	38.9 ± 1.7[5]	45.5 ± 1.3[5]	42.6 ± 1.8[5]	33.0 ± 2.7	39.1 ± 2.2	–
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	5	0	0
Week 13	10	10	10	10	5	0
Blood urea nitrogen (mg/dL)						
Week 1	19.0 ± 0.4	16.7 ± 0.9	18.0 ± 0.7	19.7 ± 1.0	22.3 ± 2.7	19.5 ± 1.3
Week 3	16.8 ± 0.4	17.4 ± 0.7	20.3 ± 0.7**	23.2 ± 0.7**	–	–
Week 13	22.3 ± 1.4	19.2 ± 0.6**	19.0 ± 1.1**	18.8 ± 1.1*	18.4 ± 1.9*	–
Creatinine (mg/dL)						
Week 1	0.48 ± 0.01	0.51 ± 0.02	0.48 ± 0.01	0.45 ± 0.02	0.52 ± 0.04	0.51 ± 0.02
Week 3	0.59 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.52 ± 0.02*	–	–
Week 13	0.55 ± 0.02	0.51 ± 0.02	0.47 ± 0.02**	0.48 ± 0.04**	0.52 ± 0.04	–
Total protein (g/dL)						
Week 1	6.1 ± 0.1	5.7 ± 0.1**	5.5 ± 0.1**	5.2 ± 0.1**	5.1 ± 0.1**	5.3 ± 0.1**
Week 3	6.0 ± 0.1	5.7 ± 0.1*	5.6 ± 0.1*	5.4 ± 0.1**	–	–
Week 13	6.6 ± 0.1	6.4 ± 0.1	6.1 ± 0.1**	5.9 ± 0.1**	5.8 ± 0.1**	–

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.2 ± 0.0**	3.1 ± 0.1**	3.0 ± 0.1**	3.1 ± 0.1**
Week 3	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.2 ± 0.1**	—	—
Week 13	3.8 ± 0.1	3.6 ± 0.1	3.6 ± 0.0	3.6 ± 0.1*	3.5 ± 0.1**	—
Alkaline phosphatase (IU/L)						
Week 1	333 ± 7	285 ± 7**	257 ± 7**	251 ± 6**	227 ± 12**	242 ± 8**
Week 3	188 ± 5	175 ± 11	120 ± 5**	85 ± 4**	—	—
Week 13	192 ± 10	171 ± 10	157 ± 12**	155 ± 13**	137 ± 9**	—
Alanine aminotransferase (IU/L)						
Week 1	26 ± 1	23 ± 1	23 ± 1	23 ± 1	29 ± 1	26 ± 2
Week 3	31 ± 2	31 ± 2	31 ± 1	29 ± 2	—	—
Week 13	36 ± 2	36 ± 3	34 ± 2	51 ± 7	35 ± 2	—
Creatine kinase (IU/L)						
Week 1	261 ± 25	309 ± 33	352 ± 60	203 ± 19	199 ± 25	199 ± 30[8]
Week 3	300 ± 36	418 ± 113	220 ± 20	170 ± 27*	—	—
Week 13	88 ± 11[2]	116 ± 26	125 ± 15	93 ± 21	114 ± 28	—
Bile acids (µmol/L)						
Week 1	6.20 ± 0.49[6]	5.57 ± 0.57[7]	8.88 ± 1.95[6]	22.70 ± 4.16**	13.33 ± 2.46**[2]	21.22 ± 3.84**[2]
Week 3	11.75 ± 2.46[5]	23.00 ± 5.21*	18.80 ± 2.93	31.80 ± 7.12**	—	—
Week 13	21.40 ± 4.08	19.30 ± 3.39	19.80 ± 3.33	21.90 ± 3.26	30.00 ± 8.91	—
Urinalysis						
n	10	10	10	10	5	0
Urine volume (mL/16 hr)						
Week 13	5.1 ± 0.3	5.6 ± 0.4	3.8 ± 0.2*	4.2 ± 0.6*	3.5 ± 0.9	—
Specific gravity						
Week 13	1.052 ± 0.002	1.047 ± 0.004	1.057 ± 0.003	1.058 ± 0.002	1.078 ± 0.010*	—
Urine pH						
Week 13	7.10 ± 0.10	7.15 ± 0.17	7.10 ± 0.10	7.15 ± 0.20	7.70 ± 0.12*	—

[1] Mean ± standard error.
[2] n=9.
[3] n=10.
[4] Not measured at this exposure level.
[5] n=8.
[6] n=5.
[7] n=7.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE						
Hematology						
n						
Week 1	10	10	9	9	10	9
Week 3	10	9	8	9	10	10
Week 13	9	10	10	10	8	0
Hematocrit (%)						
Week 1	43.6 ± 0.3	42.7 ± 0.5	43.8 ± 0.3	44.7 ± 0.7	45.9 ± 0.5**	45.0 ± 0.5*
Week 3	47.1 ± 0.7	46.7 ± 0.8	46.1 ± 0.8	43.0 ± 0.5**	42.7 ± 0.6**	38.0 ± 0.3**
Week 13	46.6 ± 0.8	47.1 ± 0.7	45.8 ± 0.7	42.9 ± 1.1*	25.3 ± 1.3**	—
Hemoglobin (g/dL)						
Week 1	14.6 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.6 ± 0.2	13.9 ± 0.2**	13.5 ± 0.1**
Week 3	15.6 ± 0.2	15.4 ± 0.2	15.2 ± 0.1	14.2 ± 0.1**	13.9 ± 0.2**	12.2 ± 0.1**
Week 13	15.5 ± 0.4	15.5 ± 0.2	15.2 ± 0.2	14.2 ± 0.3**	8.4 ± 0.4**	—
Erythrocytes (10^6/μL)						
Week 1	7.39 ± 0.05	7.09 ± 0.10*	7.31 ± 0.06	7.43 ± 0.13	7.13 ± 0.09*	7.02 ± 0.08**
Week 3	8.49 ± 0.16	8.32 ± 0.15	8.27 ± 0.16	7.64 ± 0.06**	7.47 ± 0.11**	6.29 ± 0.08**
Week 13	8.98 ± 0.15	9.27 ± 0.16	8.97 ± 0.13	8.27 ± 0.23*	3.87 ± 0.19**	—
Reticulocytes (10^6/μL)						
Week 1	0.13 ± 0.02	0.09 ± 0.01*	0.10 ± 0.02	0.06 ± 0.01**	0.01 ± 0.00**	0.02 ± 0.01**
Week 3	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.07 ± 0.02	0.27 ± 0.07
Week 13	0.09 ± 0.02[2]	0.05 ± 0.02[3]	0.05 ± 0.01	0.06 ± 0.02	0.68 ± 0.07**	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.00 ± 0.00	0.06 ± 0.04[3]
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	4.04 ± 0.92**	—
Mean cell volume (fL)						
Week 1	59.1 ± 0.3	60.4 ± 0.4*	60.1 ± 0.3*	60.1 ± 0.4*	64.3 ± 0.4**	64.0 ± 0.4**
Week 3	55.5 ± 0.5	56.2 ± 0.2	55.8 ± 0.3	56.2 ± 0.2	57.1 ± 0.2**	60.3 ± 0.3**
Week 13	51.9 ± 0.2	50.8 ± 0.2	51.1 ± 0.3	52.0 ± 0.3	65.4 ± 1.4**	—
Mean cell hemoglobin (pg)						
Week 1	19.8 ± 0.2	20.2 ± 0.2	19.7 ± 0.2	19.6 ± 0.1	19.4 ± 0.1	19.3 ± 0.2
Week 3	18.4 ± 0.2	18.5 ± 0.2	18.5 ± 0.3	18.6 ± 0.2	18.6 ± 0.2	19.4 ± 0.2**
Week 13	17.3 ± 0.4	16.7 ± 0.2	16.9 ± 0.2	17.2 ± 0.3	21.7 ± 0.5**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.6 ± 0.3	33.4 ± 0.3	32.9 ± 0.3	32.6 ± 0.3*	30.3 ± 0.3**	30.1 ± 0.3**
Week 3	33.2 ± 0.3	33.0 ± 0.3	33.1 ± 0.4	33.1 ± 0.3	32.5 ± 0.2	32.2 ± 0.4*
Week 13	33.4 ± 0.7	32.9 ± 0.4	33.1 ± 0.3	33.2 ± 0.4	33.2 ± 0.4	—
Platelets (10^3/μL)						
Week 1	895.4 ± 21.7	905.0 ± 11.2	843.8 ± 20.6	809.4 ± 15.5*	556.8 ± 24.8**	554.2 ± 17.2**
Week 3	793.5 ± 18.4	812.0 ± 16.3	643.0 ± 65.9*	580.6 ± 46.7**	604.9 ± 36.2**[4]	539.4 ± 50.2**[4]
Week 13	557.6 ± 8.0	570.2 ± 16.1	503.8 ± 9.7*	518.5 ± 9.3	581.5 ± 36.5	—
Leukocytes (10^3/μL)						
Week 1	6.35 ± 0.30	6.53 ± 0.34	6.43 ± 0.19	5.86 ± 0.20	3.45 ± 0.24**	4.40 ± 0.29**
Week 3	7.30 ± 0.41	9.19 ± 0.50	9.55 ± 0.46	7.77 ± 0.46	6.04 ± 0.53	4.18 ± 0.22**
Week 13	6.07 ± 0.35	6.08 ± 0.40	6.51 ± 0.39	6.84 ± 0.30	27.71 ± 3.95**	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	0.91 ± 0.11	1.09 ± 0.17	1.03 ± 0.10	1.07 ± 0.12	0.71 ± 0.12	1.55 ± 0.14*
Week 3	1.28 ± 0.14	1.10 ± 0.12	1.08 ± 0.18	1.26 ± 0.08	0.68 ± 0.08**	0.77 ± 0.07**[a]
Week 13	1.45 ± 0.17	1.53 ± 0.12	1.52 ± 0.17	1.35 ± 0.12	5.44 ± 1.08**	—
Lymphocytes (10^3/μL)						
Week 1	5.39 ± 0.26	5.34 ± 0.20	5.35 ± 0.22	4.66 ± 0.18*	2.71 ± 0.25**	2.79 ± 0.18**
Week 3	5.93 ± 0.36	8.01 ± 0.42	8.30 ± 0.43	6.37 ± 0.48	5.27 ± 0.47	3.43 ± 0.26**[a]
Week 13	4.47 ± 0.30	4.41 ± 0.34	4.84 ± 0.30	5.41 ± 0.31	21.96 ± 3.09**	—
Monocytes (10^3/μL)						
Week 1	0.04 ± 0.02	0.09 ± 0.04	0.02 ± 0.01	0.10 ± 0.04	0.01 ± 0.01	0.04 ± 0.03
Week 3	0.05 ± 0.02	0.06 ± 0.03	0.11 ± 0.03	0.07 ± 0.05	0.03 ± 0.02	0.03 ± 0.01[a]
Week 13	0.08 ± 0.03	0.07 ± 0.02	0.11 ± 0.05	0.05 ± 0.02	0.13 ± 0.06	—
Eosinophils (10^3/μL)						
Week 1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01[a]
Week 13	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.27 ± 0.17	—
Methemoglobin (g/dL)						
Week 3	0.20 ± 0.03	0.19 ± 0.02	0.34 ± 0.10[b]	0.25 ± 0.03[b]	0.17 ± 0.01	0.14 ± 0.02
Week 13	0.15 ± 0.05	0.15 ± 0.03	0.12 ± 0.03	0.13 ± 0.04	0.16 ± 0.06	—
Total bone marrow cellularity (10^6/femur)						
Week 1	61.8 ± 2.9	—[c]	55.8 ± 2.8[b]	73.5 ± 11.2[b]	33.5 ± 2.1**[d]	—[c]
Week 3	65.0 ± 5.7	—[c]	72.5 ± 4.9[b]	72.9 ± 5.2[b]	59.6 ± 3.9	—[c]
Week 13	46.8 ± 1.9[b]	—[c]	42.7 ± 2.8	43.7 ± 2.3	79.3 ± 2.9**[d]	—
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	10	10	10
Week 13	10	10	10	10	10	0
Blood urea nitrogen (mg/dL)						
Week 1	19.2 ± 0.6	16.7 ± 1.0	18.5 ± 0.6	17.1 ± 0.5	18.6 ± 0.8	18.4 ± 0.5
Week 3	18.7 ± 0.7	18.0 ± 0.6	18.6 ± 0.9	14.7 ± 0.6**	14.5 ± 0.4**	17.7 ± 1.5*
Week 13	22.0 ± 1.5	23.4 ± 0.8	19.3 ± 1.5	19.4 ± 0.7	21.9 ± 1.6	—
Creatinine (mg/dL)						
Week 1	0.48 ± 0.02	0.47 ± 0.02	0.53 ± 0.02	0.51 ± 0.02	0.49 ± 0.01	0.41 ± 0.01**
Week 3	0.61 ± 0.01	0.65 ± 0.02	0.62 ± 0.04	0.60 ± 0.03	0.59 ± 0.02	0.54 ± 0.02*
Week 13	0.67 ± 0.05	0.70 ± 0.03	0.67 ± 0.05	0.62 ± 0.03	0.65 ± 0.04	—
Total protein (g/dL)						
Week 1	6.1 ± 0.1	5.9 ± 0.0	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.2**	5.3 ± 0.1**
Week 3	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5.7 ± 0.1**	5.6 ± 0.1**	5.1 ± 0.1**
Week 13	6.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.2 ± 0.1**	5.9 ± 0.1**	—
Albumin (g/dL)						
Week 1	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.0	3.2 ± 0.1	3.2 ± 0.1	3.0 ± 0.0**
Week 3	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	3.2 ± 0.0**	3.1 ± 0.1**
Week 13	3.6 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.5 ± 0.1	3.4 ± 0.1*	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Week 1	664 ± 7	564 ± 12**	511 ± 15**	436 ± 10**	291 ± 10**	283 ± 7**
Week 3	316 ± 10	286 ± 4*	253 ± 5**	198 ± 4**	154 ± 6**	76 ± 4**
Week 13	132 ± 7	123 ± 7	104 ± 6**	108 ± 7**	69 ± 3**	–
Alanine aminotransferase (IU/L)						
Week 1	40 ± 2	36 ± 2	39 ± 1	38 ± 1	44 ± 2	43 ± 3
Week 3	36 ± 2	35 ± 1	37 ± 1	41 ± 2	35 ± 2	39 ± 2
Week 13	41 ± 2	41 ± 2	40 ± 2	36 ± 2	42 ± 2	–
Creatine kinase (IU/L)						
Week 1	378 ± 28	393 ± 57	478 ± 39	622 ± 96*	550 ± 50*	628 ± 155
Week 3	392 ± 44	492 ± 47	494 ± 85	563 ± 140	591 ± 67	407 ± 47
Week 13	303 ± 48	215 ± 34[a]	239 ± 24	255 ± 33	315 ± 52	–
Bile acids (µmol/L)						
Week 1	11.80 ± 1.51	13.80 ± 1.56	13.40 ± 1.70	16.10 ± 2.85	16.00 ± 2.62	18.20 ± 2.92
Week 3	11.30 ± 1.40	8.60 ± 0.60	11.50 ± 1.52	15.40 ± 4.55	27.00 ± 7.05*	44.30 ± 8.01**
Week 13	15.70 ± 2.25	14.70 ± 1.38	12.44 ± 1.51[a]	16.80 ± 2.52	24.80 ± 4.25	–
Urinalysis						
n	10	9	10	10	10	0
Urine volume (mL/16 hr)						
Week 13	3.5 ± 0.3	2.9 ± 0.2	3.4 ± 0.2	3.4 ± 0.3	2.3 ± 0.1**	–
Specific gravity						
Week 13	1.079 ± 0.002	1.088 ± 0.001**	1.084 ± 0.002	1.075 ± 0.003	1.083 ± 0.002	–
Urine pH						
Week 13	6.35 ± 0.08	6.17 ± 0.08[a]	6.35 ± 0.08	6.30 ± 0.13	5.70 ± 0.08**	–
FEMALE						
n						
Week 1	9	7	10	8	9	9
Week 3	10	10	8	11	10	10
Week 13	9	10	10	8	10	0
Hematology						
Hematocrit (%)						
Week 1	45.0 ± 1.1	44.2 ± 1.1	44.3 ± 0.5	43.5 ± 0.7	44.1 ± 0.6	43.1 ± 0.9
Week 3	45.8 ± 0.4	45.0 ± 0.5	44.8 ± 0.6	43.6 ± 0.6*	44.2 ± 0.5*	38.5 ± 0.5**
Week 13	45.8 ± 0.8	45.9 ± 0.7	45.3 ± 0.4	44.0 ± 0.5*	41.1 ± 0.9**	–
Hemoglobin (g/dL)						
Week 1	15.3 ± 0.2	15.2 ± 0.3	14.7 ± 0.1*	14.4 ± 0.2*	13.8 ± 0.1**	13.2 ± 0.3**
Week 3	15.3 ± 0.1	15.1 ± 0.1	14.9 ± 0.1**	14.4 ± 0.2**	14.3 ± 0.1**	12.0 ± 0.2**
Week 13	15.4 ± 0.2	15.2 ± 0.2	15.1 ± 0.1	14.6 ± 0.2**	13.2 ± 0.3**	–

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Hematology (continued)						
Erythrocytes (10^6/μL)						
Week 1	7.68 ± 0.21	7.47 ± 0.22	7.32 ± 0.11	7.12 ± 0.15*	7.02 ± 0.09**	6.67 ± 0.17**
Week 3	8.14 ± 0.07	7.92 ± 0.07	7.90 ± 0.10	7.52 ± 0.10**	7.59 ± 0.10**	6.21 ± 0.12**
Week 13	8.38 ± 0.14	8.41 ± 0.15	8.35 ± 0.09	8.04 ± 0.10*	5.76 ± 0.19**	—
Reticulocytes (10^6/μL)						
Week 1	0.06 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.02 ± 0.01*	0.01 ± 0.01**	0.01 ± 0.00**
Week 3	0.07 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.31 ± 0.05**
Week 13	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.03	0.05 ± 0.01	1.40 ± 0.11**	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.10 ± 0.10
Week 3	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.03 ± 0.03	0.12 ± 0.05
Week 13	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	1.46 ± 0.35**	—
Mean cell volume (fL)						
Week 1	58.7 ± 0.4	59.1 ± 0.4	60.7 ± 0.3**	61.0 ± 0.6**	62.8 ± 0.5**	64.7 ± 0.6**
Week 3	56.2 ± 0.3	56.7 ± 0.3	56.5 ± 0.4	57.8 ± 0.2**	58.2 ± 0.3**	62.0 ± 0.5**
Week 13	54.8 ± 0.3	54.5 ± 0.3	54.3 ± 0.2	54.6 ± 0.4	71.7 ± 1.2**	—
Mean cell hemoglobin (pg)						
Week 1	19.9 ± 0.3	20.3 ± 0.3	20.0 ± 0.3	20.2 ± 0.3	19.7 ± 0.2	19.8 ± 0.3
Week 3	18.8 ± 0.1	19.1 ± 0.2	18.9 ± 0.2	19.1 ± 0.2	18.9 ± 0.2	19.4 ± 0.2**
Week 13	18.4 ± 0.1	18.1 ± 0.2	18.1 ± 0.1	18.2 ± 0.2	23.1 ± 0.4**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	34.1 ± 0.5	34.4 ± 0.4	33.1 ± 0.3	33.0 ± 0.5	31.4 ± 0.4**	30.6 ± 0.5**
Week 3	33.4 ± 0.3	33.7 ± 0.4	33.3 ± 0.4	33.0 ± 0.3	32.3 ± 0.3*	31.3 ± 0.3**
Week 13	33.6 ± 0.3	33.1 ± 0.3	33.4 ± 0.2	33.2 ± 0.2	32.2 ± 0.3**	—
Platelets (10^3/μL)						
Week 1	810.6 ± 28.0	747.1 ± 18.3	633.0 ± 18.4**	528.6 ± 13.2**	377.8 ± 34.3**	257.8 ± 24.6**
Week 3	816.8 ± 19.0	723.9 ± 19.3**	666.4 ± 16.0**	612.8 ± 12.0**	562.3 ± 20.8**	248.4 ± 16.9**
Week 13	671.3 ± 17.4	622.4 ± 9.2*	611.5 ± 16.9*	542.6 ± 12.1**	360.2 ± 27.5**	—
Leukocytes (10^3/μL)						
Week 1	7.69 ± 0.42	7.26 ± 0.36	5.86 ± 0.47**	5.48 ± 0.34**	4.82 ± 0.14**	4.22 ± 0.37**
Week 3	7.18 ± 0.39	7.73 ± 0.35	8.13 ± 0.17	7.02 ± 0.52	5.92 ± 0.38	3.59 ± 0.21**
Week 13	5.57 ± 0.26	4.52 ± 0.37	5.73 ± 0.32	6.40 ± 0.40	26.20 ± 4.96**	—
Segmented neutrophils (10^3/μL)						
Week 1	1.07 ± 0.18	1.17 ± 0.18	0.89 ± 0.14	1.04 ± 0.12	1.03 ± 0.16	1.09 ± 0.19
Week 3	0.94 ± 0.05	0.86 ± 0.16	1.29 ± 0.07	0.77 ± 0.14	0.71 ± 0.10	0.53 ± 0.07**
Week 13	1.50 ± 0.13	1.09 ± 0.12	1.39 ± 0.12	1.24 ± 0.16	3.87 ± 1.09	—
Lymphocytes (10^3/μL)						
Week 1	6.49 ± 0.38	5.97 ± 0.30	4.65 ± 0.35**	4.39 ± 0.35**	3.76 ± 0.12**	3.09 ± 0.24**
Week 3	6.22 ± 0.37	6.79 ± 0.28	6.79 ± 0.21	6.25 ± 0.47	5.18 ± 0.35	3.14 ± 0.17**
Week 13	3.95 ± 0.23	3.34 ± 0.31	4.18 ± 0.25	5.00 ± 0.33*	21.55 ± 3.74**	—
Monocytes (10^3/μL)						
Week 1	0.06 ± 0.03	0.08 ± 0.04	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.02 ± 0.01	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Week 13	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.33 ± 0.20	—
Eosinophils (10^3/μL)						
Week 1	0.07 ± 0.03	0.04 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Week 3	0.02 ± 0.02	0.02 ± 0.01	0.05 ± 0.03	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Week 13	0.09 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.14 ± 0.03	0.43 ± 0.19	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Hematology (continued)						
Methemoglobin (g/dL)						
Week 3	0.12 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.11 ± 0.02
Week 13	0.17 ± 0.01	0.14 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	—
Total bone marrow cellularity (10^6/femur)						
Week 1	45.2 ± 3.0	—[d]	46.5 ± 1.8	39.9 ± 2.0	35.6 ± 3.5	—[d]
Week 3	51.6 ± 2.5	—[d]	46.8 ± 3.0[b]	48.5 ± 1.1	46.8 ± 2.1	—[d]
Week 13	32.6 ± 0.9[b]	—[d]	35.2 ± 1.4[a]	36.3 ± 1.2[a,b]	60.2 ± 3.0[a,c]	—
Clinical Chemistry						
n						
Week 1	10	10	11	9	10	10
Week 3	10	10	9	11	10	10
Week 13	10	10	10	10	10	0
Blood urea nitrogen (mg/dL)						
Week 1	19.2 ± 0.7	20.4 ± 0.7	20.5 ± 1.3	17.3 ± 0.6	15.8 ± 1.1	20.2 ± 1.1
Week 3	24.2 ± 0.8	23.5 ± 1.0	22.9 ± 0.8	23.6 ± 1.0	22.9 ± 0.8	28.4 ± 0.9[a]
Week 13	24.6 ± 0.8	23.3 ± 1.5	22.3 ± 1.3	18.8 ± 1.3[a]	23.8 ± 2.4	—
Creatinine (mg/dL)						
Week 1	0.50 ± 0.03	0.45 ± 0.03	0.53 ± 0.03	0.51 ± 0.03	0.41 ± 0.02	0.46 ± 0.02
Week 3	0.57 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.55 ± 0.02	0.50 ± 0.02[a]	0.50 ± 0.00[a,c]
Week 13	0.69 ± 0.03	0.69 ± 0.06	0.63 ± 0.03	0.60 ± 0.03	0.60 ± 0.04	—
Total protein (g/dL)						
Week 1	5.8 ± 0.2	5.7 ± 0.2	5.9 ± 0.4	5.7 ± 0.2	4.7 ± 0.2[a,c]	4.9 ± 0.2[a,c]
Week 3	6.5 ± 0.1	6.2 ± 0.1[a]	6.1 ± 0.1[a,c]	5.9 ± 0.1[a,c]	5.9 ± 0.1[a,c]	5.2 ± 0.1[a,c]
Week 13	7.0 ± 0.3	6.9 ± 0.3	6.5 ± 0.3	5.6 ± 0.3[a,c]	5.3 ± 0.3[a,c]	—
Albumin (g/dL)						
Week 1	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.2	3.2 ± 0.1	2.8 ± 0.1[a]	2.9 ± 0.1[a]
Week 3	3.8 ± 0.1	3.7 ± 0.1	3.6 ± 0.1[a]	3.4 ± 0.0[a,c]	3.5 ± 0.1[a,c]	3.3 ± 0.0[a,c]
Week 13	4.0 ± 0.1	4.0 ± 0.2	3.8 ± 0.2	3.3 ± 0.2[a]	3.2 ± 0.2[a,c]	—
Alkaline phosphatase (IU/L)						
Week 1	389 ± 28	302 ± 22[a]	347 ± 28	294 ± 14[a]	225 ± 12[a,c]	198 ± 7[a,c]
Week 3	326 ± 14	269 ± 6[a,c]	246 ± 9[a,c]	223 ± 7[a,c]	194 ± 7[a,c]	94 ± 7[a,c]
Week 13	114 ± 12	123 ± 12	108 ± 8	115 ± 5	69 ± 5[a,c]	—
Alanine aminotransferase (IU/L)						
Week 1	36 ± 1	35 ± 1	36 ± 3	40 ± 2	35 ± 2	42 ± 3[a]
Week 3	32 ± 1	32 ± 1	35 ± 1	38 ± 1[a,c]	37 ± 2[a]	47 ± 2[a,c]
Week 13	39 ± 2	40 ± 5	37 ± 2	33 ± 2	48 ± 7	—
Creatine kinase (IU/L)						
Week 1	784 ± 172	1039 ± 223	815 ± 81	954 ± 141	612 ± 45	672 ± 76
Week 3	287 ± 45	412 ± 54	549 ± 111[a,c]	407 ± 51[a]	575 ± 74[a,c]	353 ± 35[a]
Week 13	312 ± 51	522 ± 88	362 ± 99	417 ± 80	307 ± 55	—
Bile acids (µmol/L)						
Week 1	25.60 ± 5.25	19.40 ± 1.89	25.55 ± 3.54	29.00 ± 4.59	19.60 ± 3.06	27.70 ± 2.90
Week 3	10.89 ± 2.34[b]	22.11 ± 6.10[a,b]	13.00 ± 1.90	37.55 ± 6.66[a,c]	35.50 ± 4.19[a,c]	50.10 ± 5.31[a,c]
Week 13	27.30 ± 6.90	24.20 ± 8.78	16.67 ± 3.54[b]	15.30 ± 3.40	17.90 ± 2.61	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Urinalysis						
n	10	10	10	10	9	0
Urine volume (mL/16 hr)						
Week 13	2.7 ± 0.3	3.3 ± 0.4	3.2 ± 0.3	3.2 ± 0.3	2.3 ± 0.3	–
Specific gravity						
Week 13	1.065 ± 0.003	1.060 ± 0.004	1.063 ± 0.003	1.062 ± 0.004	1.075 ± 0.003	–
Urine pH						
Week 13	6.65 ± 0.18	6.70 ± 0.13	7.05 ± 0.12	7.10 ± 0.27	6.28 ± 0.12	–

[1] Mean ± standard error.
[2] n=8.
[3] n=9.
[4] Not measured at this exposure level.
[5] n=10.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE						
Hematology						
n						
Week 1	10	9	10	10	10	10
Week 3	8	9	10	10	10	10
Week 13	8	10	10	10	10	9
Hematocrit (%)						
Week 1	43.1 ± 0.8	44.6 ± 0.5	41.8 ± 1.3	42.1 ± 1.0	45.8 ± 0.8	46.4 ± 0.8*
Week 3	46.6 ± 0.6	46.2 ± 0.5	46.1 ± 0.6	47.0 ± 0.6	45.9 ± 0.3	48.0 ± 1.0
Week 13	44.8 ± 0.8	45.0 ± 0.6	44.7 ± 0.4	44.1 ± 0.7	42.3 ± 0.6*	43.4 ± 0.4
Hemoglobin (g/dL)						
Week 1	14.5 ± 0.1	14.5 ± 0.1	13.5 ± 0.3*	13.0 ± 0.2**	14.0 ± 0.2*	13.9 ± 0.2*
Week 3	15.4 ± 0.2	15.4 ± 0.2	15.2 ± 0.2	15.3 ± 0.2	14.8 ± 0.1*	15.1 ± 0.2
Week 13	15.0 ± 0.2	15.2 ± 0.1	14.9 ± 0.1	14.6 ± 0.1	14.0 ± 0.1**	13.7 ± 0.2**
Erythrocytes (10^6/μL)						
Week 1	7.26 ± 0.13	7.40 ± 0.09	6.63 ± 0.17*	6.19 ± 0.16**	6.64 ± 0.12**	6.17 ± 0.11**
Week 3	8.23 ± 0.14	8.07 ± 0.10	7.89 ± 0.13*	7.81 ± 0.10**	7.39 ± 0.06**	7.45 ± 0.15**
Week 13	8.64 ± 0.15	8.74 ± 0.10	8.54 ± 0.09	8.11 ± 0.12*	7.48 ± 0.12**	7.18 ± 0.12**
Reticulocytes (10^6/μL)						
Week 1	0.24 ± 0.02[2]	0.35 ± 0.11	0.27 ± 0.03	0.41 ± 0.05*	0.87 ± 0.11**	1.08 ± 0.13**
Week 3	0.28 ± 0.02	0.27 ± 0.02	0.23 ± 0.02	0.21 ± 0.02[2]	0.27 ± 0.02	0.26 ± 0.02
Week 13	0.14 ± 0.03	0.24 ± 0.06[2]	0.15 ± 0.02	0.18 ± 0.02	0.22 ± 0.05	0.46 ± 0.07**
Nucleated erythrocytes (10^3/μL)						
Week 1	0.05 ± 0.01	0.06 ± 0.03	0.13 ± 0.04	0.75 ± 0.17**	0.69 ± 0.26**	1.58 ± 0.42**
Week 3	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.07 ± 0.03
Week 13	0.00 ± 0.00	0.00 ± 0.00[2]	0.01 ± 0.01[2]	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02**
Mean cell volume (fL)						
Week 1	59.5 ± 0.3	60.3 ± 0.4	63.0 ± 0.7**	68.2 ± 0.7**	69.0 ± 0.6**	75.3 ± 1.5**
Week 3	56.5 ± 0.5	57.2 ± 0.2	58.4 ± 0.4**	60.1 ± 0.6**	62.3 ± 0.5**	64.4 ± 0.4**
Week 13	52.0 ± 0.4	51.5 ± 0.3	52.3 ± 0.4	54.4 ± 0.3**	56.7 ± 0.5**	60.6 ± 1.1**
Mean cell hemoglobin (pg)						
Week 1	19.9 ± 0.3	19.7 ± 0.2	20.3 ± 0.2	21.1 ± 0.3*	21.1 ± 0.2**	22.5 ± 0.3**
Week 3	18.7 ± 0.3	19.1 ± 0.1	19.2 ± 0.2	19.5 ± 0.2**	20.1 ± 0.1**	20.3 ± 0.2**
Week 13	17.4 ± 0.2	17.4 ± 0.1	17.5 ± 0.2	18.0 ± 0.2*	18.7 ± 0.3**	19.1 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.6 ± 0.4	32.6 ± 0.3	32.3 ± 0.5	31.0 ± 0.5**	30.7 ± 0.3**	30.0 ± 0.5**
Week 3	33.1 ± 0.3	33.4 ± 0.2	32.9 ± 0.2	32.5 ± 0.2	32.3 ± 0.2	31.6 ± 0.4**
Week 13	33.4 ± 0.4	33.8 ± 0.3	33.4 ± 0.3	33.1 ± 0.3	33.0 ± 0.3	31.5 ± 0.4**
Platelets (10^3/μL)						
Week 1	831.6 ± 16.5[3]	831.2 ± 16.7	775.6 ± 21.7	803.6 ± 14.0[3]	765.3 ± 15.9*	778.0 ± 6.9*
Week 3	648.1 ± 6.7	634.3 ± 12.2[4]	616.3 ± 11.7	623.4 ± 10.7	595.2 ± 17.9**	602.8 ± 19.0*[2]
Week 13	648.1 ± 6.7	634.3 ± 12.2	616.3 ± 11.7	623.4 ± 10.7	595.2 ± 17.9**	602.8 ± 19.0*
Leukocytes (10^3/μL)						
Week 1	8.43 ± 0.31	8.54 ± 0.49	7.93 ± 0.41	10.36 ± 0.26**	15.95 ± 2.87**	25.59 ± 3.33**
Week 3	9.21 ± 0.67	8.86 ± 0.42	9.31 ± 0.49	7.97 ± 0.52	8.80 ± 0.76	8.75 ± 0.62
Week 13	5.64 ± 0.32	6.40 ± 0.28	5.97 ± 0.36	6.28 ± 0.39	6.74 ± 0.31	5.64 ± 0.32

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	1.32 ± 0.17	0.99 ± 0.15	1.36 ± 0.16	2.44 ± 0.14**	2.09 ± 0.48*	3.44 ± 0.76**
Week 3	0.87 ± 0.10	0.83 ± 0.12	1.08 ± 0.15	1.00 ± 0.13	1.01 ± 0.11	1.12 ± 0.14
Week 13	1.06 ± 0.12	1.38 ± 0.09[2]	1.12 ± 0.12	1.27 ± 0.11	1.58 ± 0.20	0.98 ± 0.08
Lymphocytes (10^3/μL)						
Week 1	6.92 ± 0.41	7.39 ± 0.41	6.39 ± 0.33	7.66 ± 0.34	13.26 ± 2.33**	21.66 ± 3.04**
Week 3	8.19 ± 0.64	7.84 ± 0.48	8.07 ± 0.40	6.84 ± 0.51	7.66 ± 0.74	7.54 ± 0.55
Week 13	4.53 ± 0.36	5.18 ± 0.20[2]	4.77 ± 0.37	4.93 ± 0.46	5.11 ± 0.30	4.57 ± 0.29
Monocytes (10^3/μL)						
Week 1	0.13 ± 0.04	0.12 ± 0.04	0.09 ± 0.03	0.15 ± 0.04	0.46 ± 0.19	0.29 ± 0.08
Week 3	0.12 ± 0.04	0.14 ± 0.03	0.13 ± 0.04	0.10 ± 0.02	0.09 ± 0.04	0.07 ± 0.03
Week 13	0.04 ± 0.02	0.02 ± 0.01[2]	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.02	0.05 ± 0.03
Eosinophils (10^3/μL)						
Week 1	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.06 ± 0.02*	0.03 ± 0.02	0.13 ± 0.05*
Week 3	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01
Week 13	0.01 ± 0.01	0.03 ± 0.01[2]	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02
Methemoglobin (g/dL)						
Week 1	0.15 ± 0.03	0.15 ± 0.02	0.17 ± 0.02	0.15 ± 0.02[2]	0.17 ± 0.02	0.16 ± 0.02
Week 3	0.15 ± 0.03	0.17 ± 0.02	0.19 ± 0.03	0.22 ± 0.04	0.12 ± 0.03	0.17 ± 0.03
Week 13	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.09 ± 0.03	0.13 ± 0.04	0.10 ± 0.02
Total bone marrow cellularity (10^6/femur)						
Week 1	56.5 ± 2.5	–[3]	–	62.7 ± 2.5	71.0 ± 1.9**	71.8 ± 2.2**
Week 3	73.7 ± 3.1[4]	–	–	71.9 ± 5.5	75.3 ± 4.0	74.8 ± 3.8
Week 13	61.5 ± 2.0[4]	–	–	64.3 ± 2.0	72.3 ± 3.2**	68.1 ± 1.8**
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	10	10	10
Week 13	9	10	10	10	10	10
Blood urea nitrogen (mg/dL)						
Week 1	15.9 ± 0.4	16.4 ± 0.9	21.9 ± 0.8**	25.7 ± 1.0**	24.0 ± 1.0**	22.6 ± 1.0**
Week 3	20.5 ± 1.1	24.3 ± 1.0*	23.5 ± 0.7*	23.2 ± 0.9	25.8 ± 0.7**	26.9 ± 0.9**
Week 13	17.3 ± 0.7	19.0 ± 0.4	18.9 ± 0.8	20.2 ± 0.5**	20.1 ± 0.9**	22.6 ± 0.8**
Creatinine (mg/dL)						
Week 1	0.45 ± 0.02	0.48 ± 0.02	0.51 ± 0.03	0.46 ± 0.02	0.48 ± 0.02	0.53 ± 0.03*
Week 3	0.55 ± 0.03	0.51 ± 0.02	0.50 ± 0.04	0.51 ± 0.02	0.55 ± 0.02	0.60 ± 0.03
Week 13	0.61 ± 0.02	0.60 ± 0.02	0.61 ± 0.02	0.62 ± 0.02	0.62 ± 0.03	0.60 ± 0.02
Total protein (g/dL)						
Week 1	5.6 ± 0.1	5.7 ± 0.1	6.3 ± 0.1**	6.3 ± 0.1**	6.1 ± 0.1**	6.0 ± 0.1**
Week 3	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Week 13	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.1**	6.3 ± 0.1**	6.0 ± 0.1**

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.2 ± 0.1	3.2 ± 0.1	3.6 ± 0.1*	3.7 ± 0.1**	3.5 ± 0.1**	3.5 ± 0.0**
Week 3	3.5 ± 0.1	3.5 ± 0.0	3.4 ± 0.1	3.4 ± 0.0	3.3 ± 0.0	3.5 ± 0.1
Week 13	3.5 ± 0.0	3.5 ± 0.1	3.5 ± 0.0	3.4 ± 0.0**	3.4 ± 0.0**	3.3 ± 0.1**
Alkaline phosphatase (IU/L)						
Week 1	377 ± 12	412 ± 10	450 ± 14**	476 ± 15**	432 ± 18**	422 ± 13**
Week 3	319 ± 11	343 ± 11	304 ± 7	335 ± 11	341 ± 7	357 ± 11*
Week 13	145 ± 8	147 ± 6	144 ± 4	152 ± 7	145 ± 7	156 ± 5
Alanine aminotransferase (IU/L)						
Week 1	48 ± 2	49 ± 3	58 ± 3	58 ± 4	41 ± 3	39 ± 3*
Week 3	30 ± 1	31 ± 1	31 ± 2	31 ± 1	31 ± 2	32 ± 1
Week 13	30 ± 1	33 ± 2	32 ± 1	34 ± 2	33 ± 1	36 ± 2*
Creatine kinase (IU/L)						
Week 1	782 ± 96	544 ± 72	712 ± 101	914 ± 122	747 ± 80	767 ± 112
Week 3	416 ± 62	597 ± 114	300 ± 28	353 ± 62	440 ± 83	437 ± 66
Week 13	280 ± 50	226 ± 37	294 ± 43	226 ± 36	214 ± 20	270 ± 38
Bile acids (µmol/L)						
Week 1	11.40 ± 2.05	8.80 ± 1.04	11.70 ± 1.17	11.60 ± 0.85	9.50 ± 1.01	9.11 ± 1.06[6]
Week 3	11.44 ± 1.43[2]	18.40 ± 2.15	11.67 ± 1.72[2]	12.90 ± 2.40	10.20 ± 1.07	17.80 ± 3.15
Week 13	12.00 ± 2.35[9]	21.33 ± 6.38[7]	12.75 ± 1.85[9]	16.50 ± 4.38[9]	11.17 ± 2.43[7]	16.75 ± 4.36[9]
Urinalysis						
n	10	10	10	10	10	10
Urine volume (mL/16 hr)						
Week 13	4.8 ± 0.6	2.8 ± 0.3	2.3 ± 0.2**	2.8 ± 0.4	3.4 ± 0.5	4.4 ± 0.4
Specific gravity						
Week 13	1.046 ± 0.003	1.064 ± 0.002**	1.066 ± 0.002**	1.064 ± 0.003**	1.061 ± 0.003*	1.055 ± 0.002
Urine pH						
Week 13	6.85 ± 0.11	6.55 ± 0.12	6.85 ± 0.13	6.60 ± 0.07	6.50 ± 0.13	6.50 ± 0.11*

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE						
Hematology						
n						
Week 1	10	10	10	9	10	10
Week 3	8	8	9	10	10	10
Week 13	10	10	10	10	10	9
Hematocrit (%)						
Week 1	46.7 ± 0.4	44.8 ± 0.5**	43.8 ± 0.5**	39.3 ± 0.7**	41.1 ± 0.6**	40.5 ± 0.8**
Week 3	47.5 ± 0.9	46.7 ± 0.5	46.8 ± 0.7	46.9 ± 0.5	46.5 ± 0.7	47.4 ± 0.6
Week 13	44.8 ± 0.6	43.2 ± 0.8	42.8 ± 0.7	43.6 ± 0.7	44.4 ± 0.7	46.1 ± 0.7
Hemoglobin (g/dL)						
Week 1	15.5 ± 0.1	15.3 ± 0.1	14.6 ± 0.1**	13.1 ± 0.3**	13.4 ± 0.1**	13.3 ± 0.3**
Week 3	16.0 ± 0.3	15.6 ± 0.2	15.6 ± 0.2	15.3 ± 0.1	15.0 ± 0.2*	14.4 ± 0.1**
Week 13	14.9 ± 0.2	14.4 ± 0.2*	13.9 ± 0.2**	14.2 ± 0.2**	14.0 ± 0.2**	13.4 ± 0.2**
Erythrocytes (10^6/μL)						
Week 1	7.98 ± 0.10	7.51 ± 0.07**	7.18 ± 0.09**	6.06 ± 0.18**	5.63 ± 0.11**	5.50 ± 0.14**
Week 3	8.48 ± 0.20	8.15 ± 0.08	7.72 ± 0.15**	7.45 ± 0.11**	7.15 ± 0.12**	6.74 ± 0.10**
Week 13	8.15 ± 0.09	7.59 ± 0.15**	7.09 ± 0.14**	7.00 ± 0.12**	6.80 ± 0.11**	6.58 ± 0.14**
Reticulocytes (10^6/μL)						
Week 1	0.25 ± 0.03	0.25 ± 0.03	0.22 ± 0.04	0.55 ± 0.09*	1.11 ± 0.09**	1.15 ± 0.12**
Week 3	0.15 ± 0.02	0.17 ± 0.03	0.14 ± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.24 ± 0.06
Week 13	0.12 ± 0.02	0.17 ± 0.03	0.19 ± 0.03	0.28 ± 0.03**	0.28 ± 0.05**	0.27 ± 0.05**
Nucleated erythrocytes (10^3/μL)						
Week 1	0.05 ± 0.02	0.10 ± 0.03	0.11 ± 0.03	0.81 ± 0.19**	6.13 ± 1.19**	4.97 ± 0.99**
Week 3	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.03	0.13 ± 0.05*
Week 13	0.01 ± 0.01[2]	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.10 ± 0.03*	0.16 ± 0.04**
Mean cell volume (fL)						
Week 1	58.6 ± 0.5	59.5 ± 0.3	60.8 ± 0.3**	65.1 ± 1.0**	73.3 ± 1.3**	73.7 ± 1.4**
Week 3	56.1 ± 0.3	57.3 ± 0.5	60.7 ± 0.5**	63.1 ± 0.4**	65.1 ± 0.6**	70.5 ± 1.0**
Week 13	54.8 ± 0.3	57.0 ± 0.4**	60.5 ± 0.4**	62.4 ± 0.6**	65.3 ± 0.6**	70.1 ± 0.9**
Mean cell hemoglobin (pg)						
Week 1	19.4 ± 0.2	20.4 ± 0.1**	20.4 ± 0.2**	21.7 ± 0.3**	23.9 ± 0.4**	24.3 ± 0.5**
Week 3	18.9 ± 0.2	19.2 ± 0.3	20.3 ± 0.2**	20.6 ± 0.2**	21.0 ± 0.2**	21.3 ± 0.2**
Week 13	18.3 ± 0.2	18.9 ± 0.2	19.7 ± 0.2**	20.2 ± 0.3**	20.6 ± 0.2**	20.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.1 ± 0.3	34.2 ± 0.3	33.4 ± 0.3	33.4 ± 0.5	32.8 ± 0.4	32.9 ± 0.4
Week 3	33.6 ± 0.3	33.5 ± 0.4	33.4 ± 0.3	32.6 ± 0.2*	32.4 ± 0.4*	30.3 ± 0.4**
Week 13	33.3 ± 0.3	33.3 ± 0.3	32.6 ± 0.3	32.6 ± 0.4	31.5 ± 0.3**	29.1 ± 0.3**
Platelets (10^3/μL)						
Week 1	934.8 ± 33.7	959.0 ± 35.1	921.2 ± 49.2	1045.8 ± 45.4	1118.9 ± 29.5**	1097.7 ± 45.8*
Week 3	819.0 ± 20.8	805.4 ± 27.4	799.1 ± 28.1	754.6 ± 22.1[2]	739.4 ± 13.3**	705.8 ± 15.5**
Week 13	691.3 ± 17.0	653.9 ± 13.6	688.4 ± 25.1	578.1 ± 21.8**	495.6 ± 9.7**	495.7 ± 11.4**
Leukocytes (10^3/μL)						
Week 1	7.95 ± 0.20	7.95 ± 0.36	7.93 ± 0.54	12.07 ± 1.62	35.71 ± 3.96**	31.31 ± 3.92**
Week 3	8.56 ± 0.70	7.89 ± 0.42	7.77 ± 0.69	8.48 ± 0.66	9.96 ± 0.82	8.54 ± 0.56
Week 13	6.42 ± 0.43[2]	5.98 ± 0.35	5.99 ± 0.32	6.10 ± 0.27	6.05 ± 0.28	6.97 ± 0.55

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	1.10 ± 0.13	0.86 ± 0.11	0.74 ± 0.11	1.48 ± 0.38	4.71 ± 0.94**	4.00 ± 0.93**
Week 3	0.97 ± 0.23	1.15 ± 0.22	0.81 ± 0.16	0.90 ± 0.18	1.21 ± 0.18	0.90 ± 0.15
Week 13	0.94 ± 0.13[a]	1.20 ± 0.24	1.08 ± 0.22	1.19 ± 0.19	1.02 ± 0.15	1.29 ± 0.14
Lymphocytes (10^3/μL)						
Week 1	6.62 ± 0.26	6.92 ± 0.36	6.92 ± 0.46	10.20 ± 1.30*	30.06 ± 3.32**	26.80 ± 3.21**
Week 3	7.41 ± 0.61	6.61 ± 0.37	6.77 ± 0.57	7.38 ± 0.49	8.56 ± 0.76	7.40 ± 0.45
Week 13	5.52 ± 0.41	4.63 ± 0.20	4.81 ± 0.16	4.86 ± 0.31	4.93 ± 0.33	5.51 ± 0.47
Monocytes (10^3/μL)						
Week 1	0.14 ± 0.03	0.12 ± 0.04	0.15 ± 0.04	0.32 ± 0.08	0.66 ± 0.30	0.30 ± 0.12
Week 3	0.15 ± 0.05	0.11 ± 0.03	0.10 ± 0.04	0.16 ± 0.05	0.13 ± 0.04	0.19 ± 0.02
Week 13	0.06 ± 0.02	0.09 ± 0.03	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.03	0.08 ± 0.02
Eosinophils (10^3/μL)						
Week 1	0.04 ± 0.02	0.02 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.08 ± 0.05	0.05 ± 0.05
Week 3	0.03 ± 0.01	0.02 ± 0.02	0.07 ± 0.02	0.03 ± 0.02	0.06 ± 0.04	0.05 ± 0.02
Week 13	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
Methemoglobin (g/dL)						
Week 1	0.15 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.20 ± 0.02	0.20 ± 0.02
Week 3	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.09 ± 0.01
Week 13	0.11 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.11 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Week 1	50.6 ± 2.0	–	–	55.1 ± 1.1[a]	59.2 ± 1.6*	53.8 ± 2.6
Week 3	47.6 ± 2.0[a]	–	–	51.7 ± 1.7	49.9 ± 3.5	49.3 ± 2.6
Week 13	40.7 ± 3.8	–	–	47.3 ± 1.9*	54.5 ± 1.2**	54.5 ± 2.4**[a]
Clinical Chemistry						
n	10	10	10	10	10	10
Blood urea nitrogen (mg/dL)						
Week 1	19.6 ± 0.5	19.0 ± 1.4	20.3 ± 0.7	20.1 ± 0.9	22.2 ± 1.0	21.7 ± 0.8
Week 3	23.3 ± 0.9	21.5 ± 0.6	22.6 ± 0.9	26.1 ± 0.8*	29.1 ± 1.5**	30.9 ± 1.1**
Week 13	18.4 ± 0.5	18.7 ± 0.9	20.6 ± 0.4**	21.1 ± 1.2*	26.2 ± 0.8**	31.2 ± 1.3**
Creatinine (mg/dL)						
Week 1	0.35 ± 0.02	0.39 ± 0.01	0.33 ± 0.03	0.38 ± 0.01	0.39 ± 0.01	0.43 ± 0.02**
Week 3	0.50 ± 0.02	0.52 ± 0.01	0.53 ± 0.05	0.48 ± 0.02	0.51 ± 0.02	0.53 ± 0.02
Week 13	0.56 ± 0.02	0.56 ± 0.02	0.60 ± 0.03	0.65 ± 0.03*	0.66 ± 0.02**	0.66 ± 0.03**
Total protein (g/dL)						
Week 1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.0	5.5 ± 0.1	5.3 ± 0.1**	5.6 ± 0.1
Week 3	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1*	6.3 ± 0.1**	6.0 ± 0.1**	5.9 ± 0.0**
Week 13	6.9 ± 0.1	6.8 ± 0.1	6.4 ± 0.1**	6.3 ± 0.1**	5.8 ± 0.1**	5.7 ± 0.1**
Albumin (g/dL)						
Week 1	3.4 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.1 ± 0.1*	3.3 ± 0.1
Week 3	3.7 ± 0.1	3.8 ± 0.1	3.6 ± 0.0	3.6 ± 0.0	3.5 ± 0.1*	3.5 ± 0.0**
Week 13	3.8 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.5 ± 0.0**	3.4 ± 0.0**	3.4 ± 0.1**

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Week 1	298 ± 8	307 ± 11	323 ± 12	325 ± 13	313 ± 5	335 ± 13*
Week 3	281 ± 25	235 ± 7	249 ± 6	242 ± 7	230 ± 4	242 ± 7
Week 13	140 ± 11	142 ± 9	157 ± 10	175 ± 14	235 ± 15**	254 ± 13**
Alanine aminotransferase (IU/L)						
Week 1	24 ± 1	23 ± 0	24 ± 1	25 ± 1	28 ± 1*	31 ± 1**
Week 3	35 ± 3	29 ± 1	28 ± 2	31 ± 2	32 ± 1	32 ± 1
Week 13	27 ± 1	28 ± 1	29 ± 1	29 ± 1	31 ± 1**	33 ± 2**
Creatine kinase (IU/L)						
Week 1	542 ± 36	590 ± 57	524 ± 37	651 ± 58	644 ± 72	647 ± 55
Week 3	429 ± 102	670 ± 175	470 ± 157	431 ± 111	284 ± 51	412 ± 94
Week 13	152 ± 21	154 ± 17	166 ± 18	234 ± 30*	264 ± 22**	210 ± 27*
Bile acids (µmol/L)						
Week 1	15.22 ± 1.61[2]	23.13 ± 9.88[3]	13.89 ± 3.21[2]	15.50 ± 2.60[3]	21.20 ± 5.10	17.00 ± 3.89[2]
Week 3	12.40 ± 1.60	23.10 ± 5.84	23.00 ± 3.67*	22.70 ± 5.48	20.50 ± 3.82	22.80 ± 3.23*
Week 13	8.75 ± 1.54[3]	19.14 ± 5.26[4]	7.50 ± 1.23[7]	11.25 ± 1.84[8]	11.00 ± 1.45	13.30 ± 2.86
Urinalysis						
n	10	10	10	10	10	10
Urine volume (mL/16 hr)						
Week 13	3.8 ± 0.4	2.5 ± 0.1*	2.1 ± 0.1**	2.1 ± 0.1**	2.3 ± 0.2**	2.3 ± 0.1**
Specific gravity						
Week 13	1.055 ± 0.005	1.060 ± 0.003	1.067 ± 0.002*	1.075 ± 0.002**	1.074 ± 0.002**	1.082 ± 0.003**
Urine pH						
Week 13	6.60 ± 0.10	6.55 ± 0.12	6.65 ± 0.11	6.55 ± 0.05	6.90 ± 0.07	6.60 ± 0.07

[1] Mean ± standard error.
[2] n=9.
[3] n=8.
[4] n=10.
[5] Not measured at this exposure level.
[6] n=5.
[7] n=6.
[8] n=7.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

APPENDIX E

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	750 ppm	1500 ppm	3000 ppm
Weights (g)				
Necropsy body weight	316 ± 7	295 ± 7	260 ± 5**	214 ± 5**
Left epididymis	0.431 ± 0.012	0.427 ± 0.009	0.206 ± 0.007**	0.162 ± 0.005**
Left cauda epididymis	0.194 ± 0.006	0.189 ± 0.005	0.082 ± 0.004**	0.068 ± 0.002**
Left testis	1.494 ± 0.032	1.488 ± 0.020	0.673 ± 0.046**	0.500 ± 0.025**
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.140 ± 0.317	8.630 ± 0.331	1.790 ± 0.520**	0.000 ± 0.000**
Spermatid heads (10^7/testis)	13.69 ± 0.63	12.84 ± 0.48	1.41 ± 0.50**	0.00 ± 0.00**
Spermatid count (mean/10^{-4} mL suspension)	68.43 ± 3.17	64.20 ± 2.42	7.03 ± 2.51**	0.00 ± 0.00**
Spermatozoal measurements				
Motility (%)	98.43 ± 0.15	97.49 ± 0.39	0.00 ± 0.00**	0.00 ± 0.00**
Concentration (10^6/g caudal epididymal tissue)	755.4 ± 25.6	655.8 ± 14.1*	13.0 ± 3.4**	7.2 ± 2.2**

[1] Data presented as mean ± standard error; n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	1500 ppm	3000 ppm	4500 ppm
Necropsy body weight (g)	189 ± 4	170 ± 3**	145 ± 3**	151 ± 2**
Estrous cycle length (days)	6.72 ± 0.49[2]	7.67 ± 0.49[3]	5.17 ± 0.17[4]	7.00 ± 1.00[5]
Estrous stages (% of cycle)				
Diestrus	41.7	52.4	70.0	70.0
Proestrus	13.3	10.5	7.5	6.7
Estrus	32.5	26.7	16.7	13.3
Metestrus	12.5	10.5	5.8	10.0

[1] Data presented as mean ± standard error. For the control group and the 1500 and 3000 ppm dose groups, n=10; for the 4500 ppm dose group, n=5. Differences from the control group for estrous cycle length were not significant by Dunn's test. There is evidence to suggest that animals in the 1500 ppm (P≤0.01) and 3000 ppm (P≤0.05) dose groups differ from the controls in the relative frequency of time spent in estrous stages. Although the 4500 ppm group also appears different, Wilk's Criterion gives a P-value of 0.09. The lack of significance at this dose level may be due to increased variability and/or the small sample size (n=5).
[2] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
[3] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.
[4] Estrous cycle longer than 12 days or unclear in 7 of 10 animals.
[5] Estrous cycle longer than 12 days or unclear in 3 of 5 animals.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	2500 ppm	5000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	315 ± 5	296 ± 4**	295 ± 8*	236 ± 5**
Left epididymis	0.444 ± 0.007	0.447 ± 0.007	0.417 ± 0.008*	0.199 ± 0.008**
Left cauda epididymis	0.185 ± 0.003	0.190 ± 0.003	0.173 ± 0.004*	0.081 ± 0.003**
Left testis	1.459 ± 0.024	1.519 ± 0.020	1.410 ± 0.025	0.727 ± 0.042**
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.980 ± 0.352	9.630 ± 0.273	9.410 ± 0.376	1.610 ± 0.399**
Spermatid heads (10^7/testis)	13.12 ± 0.58	14.63 ± 0.50	13.27 ± 0.60	1.17 ± 0.31**
Spermatid count (mean/10^{-4} mL suspension)	65.58 ± 2.90	73.15 ± 2.49	66.35 ± 3.00	5.83 ± 1.57**
Spermatozoal measurements				
Motility (%)	96.55 ± 1.02	97.88 ± 0.67	97.07 ± 0.93	0.56 ± 0.44**
Concentration (10^6/g caudal epididymal tissue)	763.9 ± 23.1	658.3 ± 14.8**	669.0 ± 25.2**	27.2 ± 5.2**

[1] Data presented as mean ± standard error; n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	2500 ppm	5000 ppm	10,000 ppm
Necropsy body weight (g)	185 ± 3	177 ± 1	173 ± 3**	149 ± 1**
Estrous cycle length (days)	5.40 ± 0.15	5.83 ± 0.40[2]	5.83 ± 0.26[3]	6.50 ± 0.43*[2]
Estrous stages (% of cycle)				
Diestrus	36.7	37.3	42.5	55.0
Proestrus	15.0	11.0	15.8	10.0
Estrus	39.2	44.1	30.0	25.8
Metestrus	9.2	7.6	11.7	9.2

[1] Data presented as mean ± standard error; n=10. There is evidence that animals in the 10,000 ppm dose group differed significantly (P≤0.05, Wilk's Criterion) from the controls in the relative frequency of time spent in estrous stages. Females in this group spent more time in diestrus and less time in proestrus and estrus than did controls.
[2] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E5 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
Weights (g)				
Necropsy body weight	308 ± 6	298 ± 3	280 ± 5**	264 ± 5**
Left epididymis	0.426 ± 0.010	0.429 ± 0.004	0.405 ± 0.007*	0.405 ± 0.008*
Left cauda epididymis	0.179 ± 0.003	0.183 ± 0.003	0.176 ± 0.003	0.173 ± 0.005
Left testis	1.480 ± 0.031	1.480 ± 0.018	1.420 ± 0.018	1.420 ± 0.021
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.540 ± 0.291	9.210 ± 0.423	8.970 ± 0.374	9.290 ± 0.217
Spermatid heads (10^7/testis)	12.64 ± 0.39	13.60 ± 0.54	12.72 ± 0.52	13.26 ± 0.44
Spermatid count (mean/10^{-4} mL suspension)	63.20 ± 1.94	67.98 ± 2.69	63.60 ± 2.62	66.28 ± 2.20
Spermatozoal measurements				
Motility (%)	98.57 ± 0.08	98.31 ± 0.23	98.48 ± 0.12	98.49 ± 0.16
Concentration (10^6/g caudal epididymal tissue)	713.9 ± 16.2	633.0 ± 13.1**	656.3 ± 13.3**	617.2 ± 22.9**

[1] Data presented as mean ± standard error; n=10. Differences from the control group for cauda epididymal weights are not significant by Dunn's test; differences from the control group for left testis weights are not significant by Shirley's test; spermatozoal measurements, excluding spermatozoal concentration, are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E6 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
Necropsy body weight (g)	186 ± 4	172 ± 2**	160 ± 2**	145 ± 2**
Estrous cycle length (days)	6.50 ± 0.70[2]	6.83 ± 0.95[3]	7.57 ± 0.53[2]	5.83 ± 0.70[3]
Estrous stages (% of cycle)				
Diestrus	28.9	45.6	52.8	67.5
Proestrus	8.8	11.4	13.9	7.0
Estrus	57.9	38.6	20.4	17.5
Metestrus	4.4	4.4	13.0	7.9

[1] Data presented as mean ± standard error; n=10. Differences from the control group in estrous cycle length are not significant by Dunn's test. There is evidence that animals in the 4500 and 6000 ppm dose groups differed significantly (P≤0.01, Wilk's Criterion) from the controls in the relative frequency of time spent in estrous stages. Females in these two dosed groups spent more time in diestrus and less time in proestrus, metestrus, and estrus than did controls.
[2] Estrous cycle longer than 12 days or unclear in 3 of 10 animals.
[3] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E7 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	2000 ppm	4000 ppm	6000 ppm
Weights (g)				
Necropsy body weight	39.2 ± 0.8	39.6 ± 0.8	40.8 ± 0.8	37.8 ± 0.9
Left epididymis	0.045 ± 0.001	0.046 ± 0.002	0.042 ± 0.001	0.031 ± 0.001**
Left cauda epididymis	0.016 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.013 ± 0.001*
Left testis	0.114 ± 0.001	0.113 ± 0.003	0.097 ± 0.003**	0.025 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.440 ± 0.630	19.490 ± 0.693	16.790 ± 0.950*	1.490 ± 0.582**
Spermatid heads (10^7/testis)	2.22 ± 0.08	2.21 ± 0.11	1.63 ± 0.11**	0.04 ± 0.01**
Spermatid count (mean/10^{-4} mL suspension)	69.43 ± 2.67	69.18 ± 3.32	50.78 ± 3.29**	1.20 ± 0.46**
Spermatozoal measurements				
Motility (%)	99.29 ± 0.07	99.06 ± 0.08*	98.93 ± 0.24	0.00 ± 0.00**
Concentration (10^6/g caudal epididymal tissue)	1587.8 ± 69.03	1181.0 ± 56.29**	1077.4 ± 38.70**	335.9 ± 40.13**

[1] Data presented as mean ± standard error; n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E8 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	6000 ppm	8000 ppm	10,000 ppm
Necropsy body weight (g)	29.7 ± 0.7	27.2 ± 1.2	26.0 ± 0.9**	23.9 ± 0.9**
Estrous cycle length (days)	4.60 ± 0.22	7.17 ± 0.83[2]	5.63 ± 0.47[3]	8.50 ± 1.50[4]
Estrous stages (% of cycle)				
Diestrus	30.0	18.3	10.0	39.2
Proestrus	19.2	4.2	8.3	2.5
Estrus	34.2	70.8	62.5	50.0
Metestrus	16.7	6.7	19.2	8.3

[1] Data presented as mean ± standard error; n=10. All dose groups differed significantly from controls in the relative frequency of time spent in estrous stages (Wilk's Criterion, P≤0.01).
[2] Estrous cycle longer than 12 days or unclear in 7 of 10 animals.
[3] Estrous cycle longer than 12 days or unclear in 6 of 10 animals.
[4] Estrous cycle longer than 12 days or unclear in 8 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E9 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	5000 ppm	10,000 ppm	20,000 ppm
Weights (g)				
Necropsy body weight	38.9 ± 0.8	43.0 ± 1.1	40.5 ± 0.9	33.6 ± 0.9*
Left epididymis	0.046 ± 0.001	0.045 ± 0.001	0.047 ± 0.001	0.038 ± 0.001**
Left cauda epididymis	0.017 ± 0.001	0.018 ± 0.001	0.017 ± 0.001	0.014 ± 0.001*
Left testis	0.118 ± 0.002	0.116 ± 0.004	0.120 ± 0.002	0.091 ± 0.004**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.160 ± 0.745	19.340 ± 0.767	19.970 ± 0.961	18.710 ± 1.018
Spermatid heads (10^7/testis)	2.26 ± 0.10	2.27 ± 0.15	2.39 ± 0.10	1.72 ± 0.12*
Spermatid count (mean/10^{-4} mL suspension)	70.68 ± 3.16	70.85 ± 4.74	74.68 ± 3.18	53.68 ± 3.88*
Spermatozoal measurements				
Motility (%)	98.65 ± 0.24	98.40 ± 0.30	97.92 ± 0.25	97.35 ± 0.45*
Concentration (10^6/g caudal epididymal tissue)	1126.7 ± 55.71	1036.2 ± 94.49	1133.2 ± 63.39	1139.7 ± 91.03

[1] Data presented as mean ± standard error; n=10. Spermatozoal concentration and spermatid heads/g testis are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E10 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	5000 ppm	10,000 ppm	20,000 ppm
Necropsy body weight (g)	31.3 ± 0.8	33.2 ± 1.0	29.9 ± 1.5	27.8 ± 0.8*
Estrous cycle length (days)	4.30 ± 0.11	4.85 ± 0.15*	5.25 ± 0.23**	5.50 ± 0.47**[2]
Estrous stages (% of cycle)				
Diestrus	31.7	27.5	32.5	40.8
Proestrus	23.3	20.8	18.3	19.2
Estrus	29.2	41.7	37.5	33.3
Metestrus	15.8	10.0	11.7	6.7

[1] Data presented as mean ± standard error; n=10. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.

[2] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.

* Significantly different (P≤0.05) from the control group by Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E11 Summary of Reproductive Tissue Evaluations in Male B6C3F, Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
Weights (g)				
Necropsy body weight	40.2 ± 1.6	38.4 ± 0.9	39.1 ± 0.8	38.3 ± 0.8
Left epididymis	0.048 ± 0.001[2]	0.046 ± 0.001[2]	0.044 ± 0.001	0.046 ± 0.001[3]
Left cauda epididymis	0.018 ± 0.001[2]	0.020 ± 0.001[2]	0.017 ± 0.001	0.017 ± 0.001[3]
Left testis	0.124 ± 0.002[2]	0.113 ± 0.002**[2]	0.117 ± 0.002**	0.116 ± 0.002**[3]
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.140 ± 0.642[2]	17.810 ± 1.331[2]	17.830 ± 0.395	18.510 ± 0.645[3]
Spermatid heads (10^7/testis)	2.32 ± 0.09	2.08 ± 0.16	2.08 ± 0.03	2.16 ± 0.09
Spermatid count (mean/10^{-4} mL suspension)	72.35 ± 2.80	65.15 ± 4.86	64.90 ± 0.95	67.48 ± 2.83
Spermatozoal measurements				
Motility (%)	98.78 ± 0.11[2]	97.97 ± 0.38**[2]	98.27 ± 0.23*	92.67 ± 3.01**[3]
Concentration (10^6/g caudal epididymal tissue)	1278.2 ± 99.66[2]	1167.1 ± 46.03[2]	1394.7 ± 61.54	1437.2 ± 77.86[3]

[1] Data presented as mean ± standard error; n=10. Differences from the control group for epididymal and cauda epididymal weights are not significant by Dunn's test; spermatozoal measurements, excluding motility, are not significant by Dunn's or Shirley's test.
[2] n=9.
[3] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E12 Summary of Estrous Cycle Characterization in Female B6C3F, Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
Necropsy body weight (g)	31.1 ± 0.7	28.0 ± 0.7*	28.4 ± 0.5*	27.8 ± 0.9**
Estrous cycle length (days)	4.40 ± 0.16	4.95 ± 0.46	4.44 ± 0.18[2]	4.60 ± 0.15
Estrous stages (% of cycle)				
Diestrus	30.0	34.2	36.1	42.1
Proestrus	19.2	19.3	19.4	16.7
Estrus	36.7	36.0	31.5	33.3
Metestrus	14.2	10.5	13.0	7.9

[1] Data presented as mean ± standard error; n=10. Estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in cycle length or in the relative length of time spent in the estrous stages.
[2] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX F

Leukemia Inhibition Studies in Male F344/N Rats

LEUKEMIA INHIBITION STUDIES

Materials and Methods

Twenty male rats per dose group per isomer were used to investigate the effect of ethylene glycol ethers on the growth of Fischer leukemia cells. Ten rats per dose group received the test article only. The remaining 10 animals per dose group received the test article as well as a subcutaneous injection of 2.5×10^7 Fischer leukemia cells on the first (2-ethoxyethanol and 2-butoxyethanol) or the second (2-methoxyethanol) day of dosing. Dose levels for 2-methoxyethanol and 2-butoxyethanol were 0, 3000, or 6000 ppm and dose levels for 2-ethoxyethanol were 0, 2500, or 5000 ppm. Test articles were administered in drinking water, which was available *ad libitum*, until clinical signs of leukemia appeared in rats that were administered Fischer leukemia cells. After approximately 9 weeks, animals were killed then necropsied and the spleens and livers were weighed.

Hematology analyses were also performed on rats in the leukemia inhibition studies. At terminal sacrifice, rats were anesthetized with CO_2, and blood samples were collected from the inferior vena cava. Blood was placed in EDTA tubes, and an aliquot was used for hematologic analyses.

Results

Survival, weight gain, and water and compound consumption data, organ weights and organ-weight-to-body-weight ratios, and hematology data are presented in Tables F1-F3.

TABLE F1 Survival, Weight Gain, Water Consumption, and Compound Consumption in Male F344/N Rats at 9 Weeks in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
2-Methoxyethanol							
Not injected with leukemia cells							
0	10/10	138	272	135		22.2	
3000	10/10	139	170	31	63	19.7	349
6000	0/10[4]	139	–	–	–	15.7	677
Injected with leukemia cells							
0	9/10[5]	140	273	131		21.1	
3000	10/10	142	185	42	68	19.0	328
6000	0/10[6]	137	–	–	–	14.2	626
2-Ethoxyethanol							
Not injected with leukemia cells							
0	10/10	133	296	163		22.9	
2500	10/10	137	278	141	94	22.2	255
5000	10/10	134	273	139	92	20.0	459
Injected with leukemia cells							
0	10/10	133	277	144		22.2	
2500	10/10	137	277	140	100	20.6	231
5000	10/10	136	270	135	98	18.9	438
2-Butoxyethanol							
Not injected with leukemia cells							
0	10/10	127	258	131		21.4	
3000	10/10	131	264	133	102	18.2	246
6000	10/10	128	246	118	95	14.1	407
Injected with leukemia cells							
0	9/10[5]	127	269	141		20.9	
3000	5/10[7]	131	241	106	90	16.8	237
6000	10/10	126	239	113	89	13.8	408

[1] Number surviving at 9 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dosed group mean/control group mean) x 100.
[4] Week of death: 4 (4 rats),6 (2 rats), 7 (4 rats).
[5] Week of death: 9.
[6] Week of death: 4 (4 rats), 5 (6 rats).
[7] Week of death: 8 (1 rat), 9 (4 rats).

TABLE F2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells			
n	10	10	10
Necropsy body wt.			
2-Methoxyethanol	287 ± 4	178 ± 5**	–[3]
2-Ethoxyethanol	300 ± 6	277 ± 5*	273 ± 5**
2-Butoxyethanol	283 ± 10	281 ± 6	255 ± 4**
Liver			
2-Methoxyethanol			
Absolute	11.21 ± 0.27	6.75 ± 0.15**	–
Relative	39.11 ± 0.54	37.94 ± 0.62	–
2-Ethoxyethanol			
Absolute	11.58 ± 0.34	9.67 ± 0.18**	10.16 ± 0.33**
Relative	38.57 ± 0.63	34.94 ± 0.37**	37.32 ± 1.03
2-Butoxyethanol			
Absolute	10.68 ± 0.34	11.60 ± 0.33	10.67 ± 0.27
Relative	38.05 ± 1.55	41.36 ± 0.71*	41.87 ± 0.70**
Spleen			
2-Methoxyethanol			
Absolute	0.641 ± 0.010	0.492 ± 0.016**	–
Relative	2.24 ± 0.03	2.76 ± 0.06**	–
2-Ethoxyethanol			
Absolute	0.623 ± 0.034	0.552 ± 0.037	0.652 ± 0.008[4]
Relative	2.08 ± 0.11	1.99 ± 0.12	2.40 ± 0.04**[4]
2-Butoxyethanol			
Absolute	0.596 ± 0.016	0.674 ± 0.019*	0.910 ± 0.051**
Relative	2.13 ± 0.09	2.41 ± 0.05**	3.58 ± 0.21**

TABLE F2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Injected with leukemia cells			
n			
2-Methoxyethanol	8	9	0
2-Ethoxyethanol	9	10	10
2-Butoxethanol	6	5	10
Necropsy body wt.			
2-Methoxyethanol	264 ± 16	194 ± 4**	–
2-Ethoxyethanol	268 ± 10	272 ± 9	271 ± 6
2-Butoxyethanol	289 ± 19	228 ± 16*	232 ± 10*
Liver			
2-Methoxyethanol			
Absolute	13.64 ± 0.71	7.00 ± 0.18**	–
Relative	52.01 ± 1.44	36.10 ± 0.44**	–
2-Ethoxyethanol			
Absolute	13.85 ± 0.40	12.42 ± 0.48	10.92 ± 0.55**
Relative	51.82 ± 1.38	45.96 ± 1.99	40.48 ± 2.19**
2-Butoxyethanol			
Absolute	14.21 ± 0.80	12.65 ± 0.78	12.31 ± 0.50
Relative	49.69 ± 2.80	55.54 ± 1.46	53.22 ± 0.87
Spleen			
2-Methoxyethanol			
Absolute	10.10 ± 0.91	0.54 ± 0.03**	–
Relative	39.34 ± 4.14	2.80 ± 0.13**	–
2-Ethoxyethanol			
Absolute	9.28 ± 0.91	7.21 ± 1.25	4.13 ± 1.45**
Relative	35.67 ± 4.24	27.68 ± 5.58	15.65 ± 5.53*
2-Butoxyethanol			
Absolute	10.44 ± 0.75	8.27 ± 0.48	8.44 ± 0.26*
Relative	37.26 ± 4.35	36.91 ± 3.23	37.34 ± 1.58

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 3000, or 6000 ppm. Doses given to rats administered 2-ethoxyethanol were: 0, 2500, or 5000 ppm.

[3] Data not available due to total mortality in the 6000 ppm 2-methoxyethanol group.

[4] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test or Dunn's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test, Dunn's test, or Wilcoxon's test.

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells			
n	10	10	10
Hematocrit (%)			
2-Methoxyethanol	44.5 ± 0.5	38.5 ± 0.9**	–[3]
2-Ethoxyethanol	45.6 ± 0.6	45.0 ± 0.5[4]	45.7 ± 0.6
2-Butoxyethanol	46.0 ± 0.6[5]	44.5 ± 0.5*	43.0 ± 1.2**[4]
Hemoglobin (g/dL)			
2-Methoxyethanol	15.1 ± 0.1	12.8 ± 0.2**	–
2-Ethoxyethanol	15.0 ± 0.1	14.3 ± 0.1**[4]	13.9 ± 0.2**
2-Butoxyethanol	15.7 ± 0.2[5]	14.5 ± 0.1**	13.6 ± 0.1**[4]
Erythrocytes (10^6/μL)			
2-Methoxyethanol	8.60 ± 0.10	7.22 ± 0.20**	–
2-Ethoxyethanol	9.09 ± 0.09	8.76 ± 0.08**[4]	8.61 ± 0.14**
2-Butoxyethanol	9.22 ± 0.19[5]	8.10 ± 0.08**	7.18 ± 0.12**[4]
Reticulocytes (10^6/μL)			
2-Methoxyethanol	0.14 ± 0.01	0.19 ± 0.04	–
2-Ethoxyethanol	0.13 ± 0.03	0.19 ± 0.04[4]	0.13 ± 0.02
2-Butoxyethanol	0.05 ± 0.01[5]	0.07 ± 0.01	0.16 ± 0.03**[4]
Nucleated erythrocytes (10^3/μL)			
2-Methoxyethanol	0.05 ± 0.02	0.01 ± 0.01	–
2-Ethoxyethanol	0.07 ± 0.02	0.01 ± 0.01[4]	0.04 ± 0.02
2-Butoxyethanol	0.00 ± 0.00[5]	0.02 ± 0.01	0.23 ± 0.07**[4]
Mean cell volume (fL)			
2-Methoxyethanol	51.8 ± 0.2	53.4 ± 0.3**	–
2-Ethoxyethanol	50.1 ± 0.4	51.3 ± 0.3**[4]	53.0 ± 0.3**
2-Butoxyethanol	49.9 ± 0.5[5]	54.9 ± 0.4**	59.9 ± 1.1**[4]
Mean cell hemoglobin (pg)			
2-Methoxyethanol	17.5 ± 0.1	17.8 ± 0.3	–
2-Ethoxyethanol	16.5 ± 0.1	16.4 ± 0.1[4]	16.2 ± 0.1*
2-Butoxyethanol	17.1 ± 0.2[5]	17.9 ± 0.2**	19.0 ± 0.2**[4]
Mean cell hemoglobin concentration (g/dL)			
2-Methoxyethanol	33.8 ± 0.2	33.4 ± 0.4	–
2-Ethoxyethanol	33.0 ± 0.3	31.9 ± 0.2**[4]	30.5 ± 0.2**
2-Butoxyethanol	34.2 ± 0.2[5]	32.6 ± 0.3**	31.8 ± 0.8**[4]
Platelets (10^3/μL)			
2-Methoxyethanol	659.5 ± 7.1	325.7 ± 21.4**	–
2-Ethoxyethanol	605.1 ± 20.0	563.0 ± 8.8**[4]	520.7 ± 15.5**
2-Butoxyethanol	568.9 ± 13.0[5]	522.2 ± 15.0	575.8 ± 16.9[4]

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells (continued)			
Leukocytes ($10^3/\mu L$)			
2-Methoxyethanol	6.07 ± 0.16	2.73 ± 0.28**	–
2-Ethoxyethanol	6.38 ± 0.22	5.76 ± 0.31[4]	5.31 ± 0.22**
2-Butoxyethanol	7.38 ± 0.37[5]	6.07 ± 0.17**	6.36 ± 0.33**[4]
Segmented neutrophils ($10^3/\mu L$)			
2-Methoxyethanol	0.98 ± 0.09	0.47 ± 0.04**	–
2-Ethoxyethanol	1.18 ± 0.12	1.24 ± 0.15[4]	0.85 ± 0.09*
2-Butoxyethanol	1.29 ± 0.16[5]	1.03 ± 0.07	1.51 ± 0.17[4]
Bands ($10^3/\mu L$)			
2-Methoxyethanol	0.00 ± 0.00	0.02 ± 0.01**	–
2-Ethoxyethanol	0.05 ± 0.03	0.03 ± 0.02[4]	0.02 ± 0.01
2-Butoxyethanol	0.00 ± 0.00[5]	0.01 ± 0.01	0.02 ± 0.01[4]
Lymphocytes ($10^3/\mu L$)			
2-Methoxyethanol	4.99 ± 0.11	2.20 ± 0.26**	–
2-Ethoxyethanol	4.89 ± 0.18	4.41 ± 0.29[4]	4.34 ± 0.23
2-Butoxyethanol	5.94 ± 0.31[5]	4.89 ± 0.16*	4.75 ± 0.34**[4]
Monocytes ($10^3/\mu L$)			
2-Methoxyethanol	0.04 ± 0.01	0.01 ± 0.01	–
2-Ethoxyethanol	0.08 ± 0.02	0.05 ± 0.02[4]	0.03 ± 0.02*
2-Butoxyethanol	0.06 ± 0.02[5]	0.04 ± 0.01	0.02 ± 0.01[4]
Eosinophils ($10^3/\mu L$)			
2-Methoxyethanol	0.02 ± 0.01	0.03 ± 0.01	–
2-Ethoxyethanol	0.09 ± 0.02	0.02 ± 0.02[4]	0.05 ± 0.01
2-Butoxyethanol	0.09 ± 0.02[5]	0.09 ± 0.03	0.02 ± 0.01[4]
Methemoglobin (g/dL)			
2-Methoxyethanol	0.11 ± 0.02	0.10 ± 0.02	–
2-Ethoxyethanol	0.12 ± 0.01	0.11 ± 0.02[4]	0.12 ± 0.02
2-Butoxyethanol	0.13 ± 0.02[5]	0.10 ± 0.01	0.13 ± 0.02[4]
Undifferentiated mononuclear cells ($10^3/mL$)			
2-Methoxyethanol	0.05 ± 0.02	0.01 ± 0.00[5]	–
2-Ethoxyethanol	0.03 ± 0.02	0.01 ± 0.01[4]	0.01 ± 0.01
2-Butoxyethanol	0.00 ± 0.00[5]	0.01 ± 0.01	0.04 ± 0.02**[4]

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose 1	Dose 2	Dose 3
Injected with leukemia cells			
n	9	9	9
Hematocrit (%)			
2-Methoxyethanol	24.4 ± 2.2[4]	40.4 ± 0.9**	–
2-Ethoxyethanol	25.2 ± 3.2	33.8 ± 3.5	42.4 ± 1.9**
2-Butoxyethanol	19.5 ± 1.3[6]	20.9 ± 3.6[7]	19.0 ± 1.2[9]
Hemoglobin (g/dL)			
2-Methoxyethanol	11.0 ± 0.7[4]	13.1 ± 0.2	–
2-Ethoxyethanol	10.9 ± 0.7	12.3 ± 0.7	13.4 ± 0.3**
2-Butoxyethanol	10.8 ± 0.6[6]	9.7 ± 1.7[7]	8.3 ± 0.3**[9]
Erythrocytes (10^6/μL)			
2-Methoxyethanol	4.34 ± 0.45[4]	7.45 ± 0.15**	–
2-Ethoxyethanol	4.84 ± 0.68	6.47 ± 0.71	8.02 ± 0.36**
2-Butoxyethanol	3.91 ± 0.33[6]	3.76 ± 0.81[7]	2.88 ± 0.18**[9]
Reticulocytes (10^6/μL)			
2-Methoxyethanol	0.08 ± 0.03[4]	0.16 ± 0.01	–
2-Ethoxyethanol	0.04 ± 0.02	0.04 ± 0.01	0.10 ± 0.03
2-Butoxyethanol	0.02 ± 0.00[6]	0.03 ± 0.02[10]	0.03 ± 0.00[9]
Nucleated erythrocytes (10^3/μL)			
2-Methoxyethanol	1.16 ± 0.70[4]	0.02 ± 0.01	–
2-Ethoxyethanol	3.00 ± 1.59	0.94 ± 0.82	0.06 ± 0.04
2-Butoxyethanol	0.33 ± 0.33[6]	1.44 ± 1.44[7]	0.00 ± 0.00[9]
Mean cell volume (fL)			
2-Methoxyethanol	56.6 ± 0.9[4]	54.3 ± 0.3*	–
2-Ethoxyethanol	52.7 ± 0.8	52.8 ± 0.9	52.8 ± 0.4
2-Butoxyethanol	50.0 ± 1.1[6]	56.7 ± 2.6[7]	63.0 ± 1.8**[9]
Mean cell hemoglobin (pg)			
2-Methoxyethanol	26.1 ± 1.4[4]	17.5 ± 0.2**	–
2-Ethoxyethanol	24.2 ± 1.9	20.7 ± 2.2	16.9 ± 0.6**
2-Butoxyethanol	27.8 ± 0.9[6]	26.1 ± 1.2[7]	29.1 ± 1.2[9]
Mean cell hemoglobin concentration (g/dL)			
2-Methoxyethanol	46.1 ± 2.5[4]	32.4 ± 0.5**	–
2-Ethoxyethanol	45.7 ± 3.0	38.9 ± 3.4	32.0 ± 1.1**
2-Butoxyethanol	38.2 ± 5.1[6]	32.8 ± 2.1[7]	31.9 ± 0.6[9]
Platelets (10^3/μL)			
2-Methoxyethanol	202.5 ± 36.6[4]	364.0 ± 33.9**	–
2-Ethoxyethanol	157.0 ± 32.4	248.9 ± 48.9	382.1 ± 45.3**
2-Butoxyethanol	163.6 ± 41.2[6]	160.0 ± 40.1[7]	130.0 ± 7.9[9]

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Injected with leukemia cells (continued)			
Leukocytes ($10^3/\mu L$)			
2-Methoxyethanol	315.60 ± 71.09[4]	3.11 ± 0.23[**]	–
2-Ethoxyethanol	315.64 ± 64.70	114.68 ± 39.79[*]	20.23 ± 9.39[**]
2-Butoxyethanol[11]	325.6 ± 81.1[6]	285.0 ± 96.4[7]	243.4 ± 37.9[6]
Segmented neutrophils ($10^3/\mu L$)			
2-Methoxyethanol	4.12 ± 1.83[4]	0.51 ± 0.10	–
2-Ethoxyethanol	6.13 ± 1.31	4.09 ± 1.07	1.63 ± 0.42[**]
2-Butoxyethanol	7.04 ± 1.82[6]	9.90 ± 3.36[7]	6.28 ± 1.13[6]
Bands ($10^3/\mu L$)			
2-Methoxyethanol	0.12 ± 0.12[4]	0.01 ± 0.01	–
2-Ethoxyethanol	1.37 ± 0.99	1.13 ± 0.65	0.34 ± 0.22
2-Butoxyethanol	0.33 ± 0.33[6]	1.44 ± 1.44[7]	0.00 ± 0.00[6]
Lymphocytes ($10^3/\mu L$)			
2-Methoxyethanol	5.98 ± 2.02[4]	2.54 ± 0.18	–
2-Ethoxyethanol	21.59 ± 6.54	14.15 ± 4.53	7.65 ± 2.45[*]
2-Butoxyethanol	13.49 ± 6.00[6]	56.24 ± 39.25[7]	19.59 ± 6.86[6]
Monocytes ($10^3/\mu L$)			
2-Methoxyethanol	0.00 ± 0.00[4]	0.03 ± 0.02[4]	–
2-Ethoxyethanol	0.20 ± 0.13	0.36 ± 0.14	0.09 ± 0.06
2-Butoxyethanol	0.00 ± 0.00[6]	0.00 ± 0.00[7]	0.00 ± 0.00[6]
Eosinophils ($10^3/\mu L$)			
2-Methoxyethanol	0.00 ± 0.00[4]	0.01 ± 0.01	–
2-Ethoxyethanol	0.33 ± 0.33	0.03 ± 0.02	0.05 ± 0.03
2-Butoxyethanol	0.00 ± 0.00[6]	0.00 ± 0.00[7]	0.00 ± 0.00[6]
Methemoglobin (g/dL)			
2-Methoxyethanol	0.18 ± 0.03[4]	0.12 ± 0.03[4]	–
2-Ethoxyethanol	0.20 ± 0.04[4]	0.27 ± 0.06	0.14 ± 0.03
2-Butoxyethanol	0.34 ± 0.13[6]	0.22 ± 0.08[7]	0.34 ± 0.02[6]
Undifferentiated mononuclear cells (10^3/mL)			
2-Methoxyethanol	305.21 ± 71.29[4]	0.01 ± 0.01[**]	–
2-Ethoxyethanol	280.80 ± 65.26	92.14 ± 34.32[*]	10.22 ± 6.72[**]
2-Butoxyethanol[11]	304.7 ± 86.3[6]	217.4 ± 76.3[7]	217.6 ± 40.1[6]

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

[1] Mean ± standard error.
[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 3000, or 6000 ppm; doses administered to rats given 2-ethoxyethanol were: 0, 2500, or 5000 ppm.
[3] All rats treated with 6000 ppm 2-methoxyethanol, with and without leukemia cells, died or were killed before hematology evaluations were conducted.
[4] n=8.
[5] n=9.
[6] n=5.
[7] n=3.
[8] n=6.
[9] n=7.
[10] n=2.
[11] Data was provided to only 1 decimal place.
* Significantly different (P≤0.05) from the control group by Shirley's test or Wilcoxon's test.
** Significantly different (P≤0.01) from the control group by Shirley's test or Wilcoxon's test.

Appendix G

Genetic Toxicology

TABLE G1 Mutagenicity of 2-Methoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
TA100	0	131 ± 9.1	168 ± 12.4	148 ± 7.0	187 ± 2.3	131 ± 3.5
	100	122 ± 3.6	177 ± 7.3	141 ± 4.1	171 ± 5.4	132 ± 4.5
	333	131 ± 0.9	164 ± 5.2	128 ± 6.6	186 ± 3.8	132 ± 5.5
	1000	140 ± 9.9	179 ± 7.3	148 ± 6.4	193 ± 12.3	119 ± 10.9
	3333	131 ± 6.7	167 ± 5.9	131 ± 6.4	168 ± 5.5	123 ± 1.5
	10,000	129 ± 9.7	155 ± 6.1	127 ± 9.5	165 ± 1.2	130 ± 5.4
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control[3]		530 ± 29.1	1212 ± 79.3	520 ± 2.6	1599 ± 52.9	1582 ± 57.7
TA1535	0	25 ± 3.4	11 ± 1.9	12 ± 2.3	14 ± 3.2	17 ± 3.7
	100	18 ± 1.2	8 ± 1.3	12 ± 0.9	9 ± 1.2	11 ± 2.4
	333	21 ± 1.7	10 ± 1.3	12 ± 2.7	10 ± 0.5	16 ± 1.0
	1000	19 ± 4.9	12 ± 0.7	11 ± 2.4	11 ± 0.3	15 ± 1.2
	3333	22 ± 4.0	9 ± 1.9	10 ± 2.2	12 ± 0.9	13 ± 3.6
	10,000	18 ± 3.0	10 ± 1.7	11 ± 2.2	10 ± 1.2	14 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		445 ± 7.8	62 ± 4.3	251 ± 11.3	243 ± 5.2	178 ± 11.5
TA97	0	128 ± 6.1	128 ± 4.7	135 ± 1.3	132 ± 4.8	128 ± 5.2
	100	140 ± 4.2	155 ± 13.5	142 ± 4.4	128 ± 4.1	138 ± 2.8
	333	140 ± 12.3	119 ± 4.9	127 ± 2.5	138 ± 6.8	129 ± 4.0
	1000	148 ± 4.2	137 ± 8.7	128 ± 0.9	134 ± 4.1	141 ± 4.3
	3333	128 ± 3.5	129 ± 2.6	134 ± 5.8	142 ± 5.5	137 ± 0.9
	10,000	128 ± 5.1	122 ± 7.8	128 ± 1.2	137 ± 4.3	121 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		542 ± 14.7	1528 ± 9.5	2127 ± 86.6	2774 ± 68.6	1058 ± 75.8
TA98	0	30 ± 3.4	29 ± 2.1	42 ± 2.3	36 ± 3.5	41 ± 1.3
	100	31 ± 0.9	27 ± 1.5	40 ± 2.6	34 ± 3.8	46 ± 2.4
	333	33 ± 2.1	31 ± 3.7	35 ± 1.7	27 ± 4.2	47 ± 1.9
	1000	29 ± 2.4	32 ± 3.5	43 ± 1.2	29 ± 3.3	35 ± 6.5
	3333	30 ± 1.5	41 ± 2.3	32 ± 2.6	34 ± 2.3	45 ± 1.2
	10,000	29 ± 1.3	31 ± 0.9	38 ± 1.9	29 ± 2.7	46 ± 5.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		363 ± 28.2	385 ± 15.4	273 ± 9.7	559 ± 13.2	305 ± 17.3

[1] Study performed at Microbiological Associates, Inc. The detailed protocol is presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± the standard error from 3 plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE G2 Mutagenicity of 2-Ethoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+ 10% hamster S9 Trial 1	+ 10% hamster S9 Trial 2	+ 10% hamster S9 Trial 3	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2
TA100	0	152 ± 6.2	145 ± 2.9	133 ± 9.8	132 ± 9.6	139 ± 7.4	138 ± 10.1	146 ± 9.9
	100	136 ± 5.7	156 ± 5.6	123 ± 10.4	117 ± 5.9	126 ± 5.4	140 ± 6.1	155 ± 1.3
	333	138 ± 2.9	145 ± 8.1	130 ± 11.6	134 ± 5.2	131 ± 6.3	138 ± 2.7	137 ± 4.1
	1000	142 ± 4.3	136 ± 4.9	119 ± 7.5	131 ± 7.1	114 ± 9.7	149 ± 9.7	157 ± 7.2
	3333	133 ± 4.4	134 ± 9.0	129 ± 6.9	118 ± 6.7	129 ± 4.6	140 ± 2.3	139 ± 6.5
	10,000	147 ± 3.8	139 ± 9.3	127 ± 6.2	114 ± 8.8	134 ± 7.5	138 ± 3.7	149 ± 5.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[3]		2113 ± 13.0	1355 ± 13.4	125 ± 4.7	1339 ± 21.1	2408 ± 9.7	1429 ± 40.9	1119 ± 28.1
TA1535	0	29 ± 1.2	24 ± 4.0	11 ± 2.4	10 ± 1.7	16 ± 2.3	11 ± 2.4	16 ± 2.2
	100	31 ± 0.9	30 ± 3.6	11 ± 1.3	8 ± 1.3	16 ± 2.6	12 ± 1.9	13 ± 1.2
	333	23 ± 3.7	30 ± 3.1	13 ± 2.5	10 ± 1.9	14 ± 3.2	15 ± 3.6	17 ± 1.2
	1000	27 ± 2.2	29 ± 2.3	11 ± 0.9	10 ± 1.2	15 ± 1.2	11 ± 0.3	15 ± 2.3
	3333	28 ± 2.2	23 ± 4.1	13 ± 2.5	7 ± 0.9	15 ± 1.8	12 ± 1.2	15 ± 2.2
	10,000	22 ± 2.0	27 ± 0.6	10 ± 1.3	8 ± 1.2	15 ± 2.3	11 ± 2.1	15 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1562 ± 70.0	1132 ± 19.2	10 ± 0.7	123 ± 0.8	156 ± 10.0	106 ± 12.6	45 ± 6.2
TA1537	0	12 ± 3.5	12 ± 2.7	11 ± 1.7	9 ± 0.9	13 ± 1.7	8 ± 0.7	11 ± 2.3
	100	10 ± 0.3	10 ± 2.1	9 ± 0.7	8 ± 1.5	10 ± 3.3	9 ± 1.5	15 ± 0.7
	333	13 ± 1.5	9 ± 2.9	12 ± 1.5	8 ± 1.7	9 ± 1.0	9 ± 3.1	16 ± 0.3
	1000	8 ± 2.9	7 ± 2.1	13 ± 3.8	10 ± 2.0	11 ± 3.2	11 ± 3.0	12 ± 0.6
	3333	6 ± 1.2	10 ± 2.2	10 ± 1.7	8 ± 1.2	10 ± 3.0	10 ± 1.2	11 ± 2.3
	10,000	10 ± 1.5	11 ± 1.5	12 ± 1.9	11 ± 1.5	13 ± 1.3	8 ± 1.5	16 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		390 ± 21.0	255 ± 37.8	10 ± 1.5	146 ± 7.1	221 ± 23.7	149 ± 7.0	78 ± 10.4
TA98	0	24 ± 3.1	21 ± 0.3	35 ± 2.2	35 ± 0.7	33 ± 1.7	32 ± 3.5	41 ± 2.2
	100	25 ± 2.1	21 ± 1.2	33 ± 6.8	36 ± 4.5	33 ± 2.3	30 ± 1.2	37 ± 1.7
	333	24 ± 4.0	19 ± 1.9	40 ± 4.0	30 ± 3.1	33 ± 3.8	30 ± 2.9	39 ± 3.6
	1000	20 ± 1.3	26 ± 4.4	32 ± 5.6	34 ± 4.8	37 ± 4.4	35 ± 4.3	28 ± 1.2
	3333	25 ± 0.7	19 ± 0.3	27 ± 3.5	31 ± 3.5	35 ± 1.2	32 ± 6.0	39 ± 6.4
	10,000	30 ± 4.7	25 ± 4.0	32 ± 4.6	31 ± 4.7	33 ± 5.7	32 ± 2.5	35 ± 4.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1869 ± 72.9	1323 ± 18.3	21 ± 2.3	1197 ± 34.9	2187 ± 67.0	1092 ± 26.1	912 ± 38.4

[1] Study performed at EG&G Mason Research Institute. The detailed protocol is presented in Zeiger *et al.* (1985). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± the standard error from 3 plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE G3 Mutagenicity of 2-Butoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 5%	+ rat S9 10%	+ rat S9 30%
TA100	0	164 ± 5.5	153 ± 9.5	161 ± 7.5		172 ± 6.1	111 ± 2.8
	100	156 ± 11.6	157 ± 3.2	166 ± 3.2		155 ± 8.3	127 ± 12.1
	333	157 ± 7.1	161 ± 12.7	157 ± 17.5		167 ± 8.5	99 ± 5.0
	1000	165 ± 17.0	156 ± 1.8	150 ± 1.5		169 ± 4.7	96 ± 3.7
	3333	166 ± 2.6	151 ± 4.8	151 ± 13.3		156 ± 3.3	150 ± 2.3
	10,000	112 ± 7.8	132 ± 15.5	149 ± 12.1		142 ± 9.2	119 ± 4.0
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control[3]		428 ± 30.9	930 ± 56.0	731 ± 52.2		471 ± 17.0	621 ± 9.8
TA1535	0	30 ± 4.9	14 ± 0.9	14 ± 2.6		12 ± 0.3	13 ± 0.3
	100	39 ± 0.3	13 ± 0.6	12 ± 1.5		10 ± 4.7	14 ± 1.5
	333	33 ± 4.3	12 ± 1.8	14 ± 3.4		11 ± 1.3	14 ± 0.3
	1000	25 ± 3.2	8 ± 1.8	12 ± 2.0		11 ± 0.7	12 ± 2.5
	3333	25 ± 3.2	13 ± 4.0	12 ± 0.6		8 ± 0.9	10 ± 0.3
	10,000	22 ± 2.5	7 ± 2.5	10 ± 1.0		11 ± 0.6	10 ± 2.0
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control		585 ± 26.0	203 ± 10.1	698 ± 29.8		195 ± 16.0	186 ± 4.5
TA1537	0	11 ± 3.2		13 ± 1.5			13 ± 3.4
	100	13 ± 2.6		14 ± 2.1			11 ± 1.8
	333	13 ± 1.9		7 ± 1.2			8 ± 1.2
	1000	10 ± 1.9		12 ± 1.5			9 ± 3.3
	3333	9 ± 1.3		10 ± 2.3			12 ± 4.1
	10,000	14 ± 2.4		11 ± 1.3			7 ± 0.6
Trial summary		Negative		Negative			Negative
Positive control		742 ± 61.5		64 ± 3.8			49 ± 2.9
TA97	0	180 ± 15.1	171 ± 10.4	180 ± 3.0	183 ± 11.9	178 ± 6.6	198 ± 11.3
	100	178 ± 4.9	170 ± 18.0	210 ± 8.2	177 ± 8.9	196 ± 8.5	215 ± 13.2
	333	190 ± 8.4	169 ± 3.0	197 ± 5.2	187 ± 2.0	195 ± 16.5	210 ± 5.0
	666				154 ± 9.5	195 ± 15.1	170 ± 15.2
	1000	214 ± 3.7	204 ± 6.9	193 ± 3.3	169 ± 10.3	184 ± 6.4	149 ± 11.4
	1666				161 ± 19.1	166 ± 22.1	178 ± 2.9
	3333	190 ± 2.7	172 ± 11.5	164 ± 0.7			
	10,000	181 ± 1.8	148 ± 10.3	130 ± 4.1			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		799 ± 76.2	285 ± 14.7	456 ± 20.5	494 ± 22.3	355 ± 13.1	308 ± 8.8

TABLE G3 Mutagenicity of 2-Butoxyethanol in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 5%	+ rat S9 10%	+ rat S9 30%
TA98	0	25 ± 2.3	19 ± 0.6	32 ± 1.9		34 ± 1.9	40 ± 0.6
	100	24 ± 3.0	26 ± 1.0	22 ± 3.4		33 ± 3.5	35 ± 4.7
	333	22 ± 2.5	20 ± 0.9	28 ± 2.0		22 ± 3.4	37 ± 5.7
	1000	25 ± 5.0	27 ± 0.6	28 ± 0.9		24 ± 3.2	34 ± 1.2
	3333	21 ± 2.8	26 ± 2.9	30 ± 1.2		27 ± 1.7	34 ± 2.3
	10,000	11 ± 1.5[4]	21 ± 4.3	27 ± 1.2		23 ± 2.8	42 ± 1.2
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control[3]		488 ± 48.6	933 ± 29.6	528 ± 35.3		355 ± 7.4	135 ± 6.9

[1] Study performed at SRI International. The detailed protocol is presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.
[2] Revertants are presented as mean ± the standard error from 3 plates.
[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[4] Slight toxicity.

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol[1]

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Distilled water						
		72	86	150	69	
		86	106	163	63	
		92	94	130	47	
		101	114	127	42	55
Methyl methanesulfonate (µg/mL)						
	5	54	48	636	393	
		49	31	716	489	441*
2-Ethoxyethanol (µL/mL)						
	1	65	84	67	34	
		72	107	83	39	
		95	118	106	37	37
	1.5	70	106	73	35	
		64	88	72	38	
		59	85	68	38	37
	2	51	62	60	39	
		73	101	80	36	
		70	86	84	40	39
	3	73	109	105	48	
		57	71	123	72	
		74	103	105	47	56
	4	80	90	106	44	
		98	126	105	36	
		91	122	92	34	38
	5	63	110	80	42	
		65	78	106	55	
		59	113	94	53	50
Trial 2						
Distilled water						
		89	85	129	48	
		87	109	85	32	
		87	98	92	35	
		86	99	102	40	39
Methyl methanesulfonate (µg/mL)						
	5	31	23	468	503	
		43	33	466	363	433*

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency	Relative Total Growth	Mutant Count	Mutant Fraction	Average Mutant
-S9 (continued)						
Trial 2 (continued)						
2-Ethoxyethanol (μL/mL)						
	1	60	73	40	22	
		64	62	49	25	24
	1.5	76	66	92	40	
		73	72	80	36	
		74	74	85	38	38
	2	53	78	63	40	
		65	74	58	30	
		54	54	72	45	38
	3	67	84	107	53	
		50	63	72	48	
		63	52	105	56	52
	4	70	77	82	39	
		81	62	96	40	
		70	81	106	50	43
	5	58	56	71	41	
		62	70	93	50	
		69	51	62	30	40
+S9						
Trial 1						
Distilled water						
		95	89	104	37	
		101	109	89	29	
		87	98	60	23	
		95	104	91	32	30
Methylcholanthrene (μg/mL)						
	2.5	47	16	654	462	
		50	15	690	462	
		51	15	727	472	465[a]
2-Ethoxyethanol (μL/mL)						
	0.5	73	68	103	47	
		96	87	87	30	
		90	92	76	28	35
	1	92	84	83	30	
		72	85	72	33	
		85	88	82	32	32
	2	87	74	105	40	
		101	79	118	39	
		96	78	111	38	39
	3	104	77	101	32	
		107	91	120	37	
		107	69	137	43	38
	4	77	84	121	52	
		72	78	103	48	
		88	71	117	44	48[a]
	5	113	66	173	51	

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Distilled water						
		103	149	116	38	
		66	73	77	39	
		82	82	90	37	
		103	96	78	25	35
Methylcholanthrene (µg/mL)						
	2.5	31	15	783	851	
		53	28	800	506	
		51	18	748	489	615[a]
2-Ethoxyethanol (µL/mL)						
	0.5	87	103	65	25	
		82	100	95	39	
		92	137	69	25	25
	1	104	122	76	24	
		82	89	65	26	
		92	137	69	25	25
	2	94	117	109	39	
		91	122	57	21	
		85	106	73	29	29
	3	75	89	99	44	
		114	169	125	37	
		87	125	105	40	40
	4	97	108	135	46	
		86	100	126	49	
		75	78	106	47	47
	5	71	83	128	60	
		96	81	131	46	
		107	104	125	39	48
Trial 3						
Ethanol		84	69	75	30	
		93	157	96	34	
		83	88	94	38	
		85	86	91	36	34
Methylcholanthrene (µg/mL)						
	2.5	40	11	723	603	
		27	7	473	577	
		45	8	858	633	604[a]

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 3 (continued)						
2-Ethoxyethanol (μL/mL)						
	0.5	63	77	89	47	
		84	101	100	40	
		64	54	96	50	46
	1	78	83	85	37	
		98	91	111	38	
		71	91	109	51	42
	2	114	90	122	36	
		65	74	94	49	42
	3	90	61	163	60	
		80	75	119	49	
		81	84	121	50	53*
	4	85	111	106	42	
		85	48	126	50	46
	5	96	101	115	40	
		92	111	138	50	
		55	30	110	67	52*

[1] Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail in Myhr *et al.* (1985). All doses were tested in triplicate; the average of the 3 tests is presented in the table.

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

* Significant positive response ($P \leq 0.05$).

TABLE G5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Ethoxyethanol[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary: Positive								
Medium		50	1048	444	0.42	8.9	25.5	
Mitomycin-C	0.005	50	1036	1583	1.52	31.7	25.5	260.66
2-Ethoxyethanol	951	50	1039	480	0.46	9.6	25.5	9.04
	3170	50	1041	676	0.64	13.5	25.5	53.28*
	9510	50	1017	1368	1.34	27.4	25.5	217.50*
								P<0.001[3]
+S9								
Trial 1								
Summary: Positive								
Medium		50	1041	415	0.39	8.3	25.5	
Cyclophosphamide	1.5	50	1040	1408	1.35	28.2	25.5	239.61
2-Ethoxyethanol	951	50	1042	454	0.43	9.1	25.5	9.29
	3170	50	1041	517	0.49	10.3	25.5	24.58*
	9510	50	1031	609	0.59	12.2	25.5	48.17*
								P<0.001

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol and these data are presented in Galloway *et al.* (1987).
[2] SCE's/chromosome of culture exposed to 2-ethoxyethanol relative to those of culture exposed to solvent.
[3] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.
* Positive (>20% increase over solvent control).

TABLE G6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Butoxyethanol[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary: Equivocal								
Medium		50	1016	418	0.41	8.4	26.0	
Mitomycin-C								
	0.001	50	1017	568	0.55	11.4	26.0	35.75
	0.010	5	103	150	1.45	30.0	26.0	253.96
2-Butoxyethanol								
	1510	50	1004	410	0.40	8.2	31.0[3]	-0.74
	2220	50	998	453	0.45	9.1	31.0[3]	10.33
	3000	50	1013	496	0.48	9.9	31.0[3]	19.01
								P=0.001[4]
Trial 2								
Summary: Negative								
Medium		50	1027	485	0.47	9.7	26.0	
Mitomycin-C								
	0.001	50	1015	626	0.61	12.5	26.0	30.60
	0.010	5	102	202	1.98	40.4	26.0	319.36
2-Butoxyethanol								
	2500	50	1007	531	0.52	10.6	36.0[3]	11.66
	3000	50	1009	541	0.53	10.8	36.0[3]	13.54
	3500	50	1007	551	0.54	11.0	36.0[3]	15.86
								P=0.010
+S9								
Trial 1								
Summary: Negative								
Medium		50	1006	491	0.48	9.8	26.0	
Cyclophosphamide								
	0.4	50	1038	705	0.67	14.1	26.0	39.16
	2.0	5	102	128	1.25	25.6	26.0	157.11
2-Butoxyethanol								
	500	50	1019	485	0.47	9.7	26.0	-2.48
	1670	50	1015	479	0.47	9.6	26.0	-3.31
	5000	50	1026	497	0.48	9.9	26.0	-0.75
								P=0.563

TABLE G6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Butoxyethanol (continued)

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol and these data are presented by Galloway *et al.* (1987).
[2] SCEs/chromosome of culture exposed to 2-butoxyethanol relative to those of culture exposed to solvent.
[3] Because 2-butoxyethanol induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.
[4] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.

TABLE G7 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Ethoxyethanol[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 — Harvest time: 10.5 hours Summary: Positive					Trial 1 — Harvest time: 10.5 hours Summary: Negative				
Medium					Medium				
	100	2	0.02	2.0		100	1	0.01	1.0
Triethylenemelamine					Cyclophosphamide				
0.75	100	28	0.28	21.0	25.0	80	38	0.48	26.0
2-Ethoxyethanol					2-Ethoxyethanol				
4780	100	3	0.03	3.0	4780	100	1	0.01	1.0
6830	100	12	0.12	11.0[2]	6830	100	1	0.01	1.0
9510	100	15	0.15	13.0[2]	9510	100	1	0.01	1.0
				P<0.001					P=0.500

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol is found in Galloway *et al.* (1987).
[2] Clear increase in complex aberrations.
[3] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

TABLE G8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Butoxyethanol[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 — Harvest time: 10.5 hours					**Trial 1 — Harvest time: 12.5 hours**				
Summary: Negative					Summary: Negative				
Medium					Medium				
	200	7	0.04	3.5		200	6	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.25	200	22	0.11	10.5	7.5	200	20	0.10	8.0
0.75	25	14	0.56	36.0	37.5	25	10	0.40	36.0
2-Butoxyethanol					2-Butoxyethanol				
2513	200	3	0.02	1.5	2513	100	1	0.01	1.0
3750	200	2	0.01	1.0	3750	200	8	0.04	3.5
5000	100	0	0.00	0.0	5000	200	6	0.03	3.0
				P=0.991					P=0.368
Trial 2 — Harvest time: 20.5 hours[2]									
Summary: Weak positive									
Medium									
	100	0	0.00	0.0					
Mitomycin-C[3]									
0.05	25	22	0.88	36.0					
0.08	200	16	0.08	5.0					
2-Butoxyethanol									
2513	100	4	0.04	3.0					
3750	100	1	0.01	1.0					
5000	100	8	0.08	7.0*					
				P=0.007					

TABLE G8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Butoxyethanol (continued)

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 3 — Harvest time: 20.7 hours[2]									
Summary: Negative									
Medium									
	100	1	0.01	1.0					
Mitomycin-C									
0.05	100	27	0.27	22.0					
0.08	25	15	0.60	40.0					
2-Butoxyethanol									
4500	100	1	0.01	1.0					
4700	100	3	0.03	3.0					
5000	100	2	0.02	2.0					
				P=0.215					

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol is found in Galloway *et al.* (1987).
[2] Because of significant 2-butoxyethanol-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest.
[3] Control doses were switched.
* Positive ($P \leq 0.05$).

TABLE G9 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 2-Ethoxyethanol[1]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[2]	
Test 1								
Feeding	5110	4	14	2/2019	2/2004	3/1997	7/6020	(0.12%)
	0			1/2029	2/1959	0/1924	3/5912	(0.05%)
Injection	5170	10	0	0/2057	3/2055	1/1991	4/6103	(0.07%)
	0			0/2026	1/2004	1/2029	2/6059	(0.03%)
Test 2								
Feeding	20,000	2	0	2/1946	1/2451	3/1900	6/6297	(0.10%)
	0			2/2033	2/2259	1/2082	5/6374	(0.08%)
Injection	50,000	2	0	0/1969	2/1900	1/1929	3/5798	(0.05%)
	0			0/1950	0/2018	0/1897	0/5865	(0.00%)

[1] A detailed description of the protocol and the data from Test 1 are found in Valencia *et al.* (1985). Protocol and data from Test 2 are found in Mason *et al.* (1992). Results were not significant at the 5% level (Margolin *et al.*, 1983).

[2] Combined total number of lethal mutations/number of X chromosomes tested for 3 mating trials.

Triage of 8(e) Submissions

Date sent to triage: _____ NON-CAP (CAP)

Submission number: 12709A TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX (SBTOX) SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

(STOX) CTOX EPI RTOX (GTOX)

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: 0 1 (2) **pages** 1 **pages** _____

Notes:

Contractor reviewer : JW **Date:** 1/24/96

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0192-12709 SEQ. A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Clorox Company

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0676) CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/USERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 01/04/92 OTS DATE: 01/05/92 CSRAD DATE: 02/09/95

CHEMICAL NAME: | CASE
~~Butyl~~ Cellosolve, butyl ether | 111-76-2
~~methyl~~ Cellosolve, methyl ether | 109-86-4
Cellosolve | 110-80-5

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
(0202)	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04 ok.
[redacted]	MUTA (IN VITRO)	[redacted]
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
(0213)	SUB ACUTE TOX (ANIMAL)	01 02 04
(0214)	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
(0224)	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
[redacted]	METAB/PHARMACO (ANIMAL)	[redacted]
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
(0244)	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
(0248)	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA: NON-CBI INVENTORY
(YES)
CAS SR NO
IN TRIAMINI

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
RAT
MUS
WHITECO
in vitro
drosophila

TOXICOLOGICAL CONCERN:
(LOW) 2-methoxyethanol (mice)
2-ethoxyethanol
2-butoxyethanol
MED → 2-methoxyethanol (rats)
HIGH

USE: solvent, chemical intermediate

PRODUCTION:

COMMENTS

12709A

M

2-Methoxyethanol: Subacute oral toxicity in rats is of moderate concern. Male and female F344 rats (5/sex/dose) received 0, 200, 400, 600, 1,000, or 1,200 mg/kg/day for two weeks. There were no deaths. Decreased thymus weight (dose-related) occurred in males and females and decreased testis weights (dose-related) occurred in males. Necropsy revealed the following in 1,000- and 1,200-mg/kg/day females and 1,200-mg/kg/day males: hemorrhage and edema of the forestomach mucosa; focal necrosis and ulceration of the forestomach squamous epithelium; mild hyperplasia of the forestomach squamous mucosa; and sinusoidal congestion, hemorrhage, and erythrophagocytosis of the mesenteric lymph nodes. In addition, testicular degeneration occurred in males at ≥400 mg/kg/day and consisted of moderate to marked loss of germinal epithelium and multinucleated spermatid giant cells and cell debris in the lumen of the seminiferous tubules.

L

2-Methoxyethanol: Subacute oral toxicity in mice is of low concern. Male and female B6C3F1 mice (5/sex/dose) received 0, 200, 400, 600, 1,000, or 1,200 mg/kg/day for two weeks. There were no deaths. Decreased thymus weight and testis weight (dose-related) occurred in males. Decreased thymus weight also occurred in 1,000- and 1,200-mg/kg/day females. There were no gross or microscopic pathological effects.

L

2-Ethoxyethanol: Subacute oral toxicity in rats and mice is of low concern. Male and female F344 rats and B6C3F1 mice (5/sex/dose) received 0, 300, 600, 900, 1,500, or 2,500 mg/kg/day for two weeks. No rats died; one 900-mg/kg/day male mouse died. Decreased thymus weight (dose-related) occurred in male and female rats and decreased testis weight (dose-related) occurred in male rats. Decreased testis weight also occurred in high-dose male mice. Testicular degeneration occurred in male rats at 1,500 and 2,500 mg/kg/day and consisted of moderate to marked loss of germinal epithelium and multinucleated spermatid giant cells and cell debris in the lumen of the seminiferous tubules. There were no gross or microscopic pathological effects in mice.

L

2-Butoxyethanol: Subacute oral toxicity in rats and mice is of low concern. Male and female F344 rats and B6C3F1 mice (5/sex/dose) received 0, 100, 150, 250, 400, or 650 mg/kg/day for two weeks. There were no deaths in rats or mice. Decreased thymus weight occurred in 650-mg/kg/day female rats and 400- and 650-mg/kg/day male mice. There were no gross or microscopic pathological effects in rats or mice.

61

12c) 8EHQ-92-12709: Rank - low.

Chemical: 2-butoxyethanol (butyl cellosolve: CAS# 111-76-2).

Draft NTP Technical Report on Toxicity Studies on Ethylene Glycol Ethers: 2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol (CAS Nos. 109-86-4, 110-80-5, 111-76-2) Administered in Drinking Water to F344/N Rats and B6C3F_1 Mice, US DHHS, dated December 1-2, 1992: Negative for gene mutations in Salmonella typhimurium in strains TA97, TA98, TA100, TA1535 and TA1537 both without and with metabolic.

Negative for chromosome mutations (aberrations) in Chinese hamster ovary (CHO) cells in vitro both without and with metabolic activation.

Does not induce DNA effects in the form of sister chromatid exchanges (SCEs) in CHO cells in vitro both without and with metabolic activation.

NOTE
Although the mutagenicity data were negative, this 8e was submitted because of toxicity information for other endpoints. These were not addressed by this reviewer.

12a) .09 8EHQ-92-12709: Rank - low.

Chemical: 2-methoxyethanol (methyl cellosolve: CAS# 109-86-4).

Draft NTP Technical Report on Toxicity Studies on Ethylene Glycol Ethers: 2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol (CAS Nos. 109-86-4, 110-80-5, 111-76-2) Administered in Drinking Water to F344/N Rats and B6C3F$_1$ Mice, US DHHS, dated December 1-2, 1992: Negative for gene mutations in Salmonella typhimurium in strains TA97, TA98, TA100, TA1535 and TA1537 both without and with metabolic.

NOTE
Although the mutagenicity data were negative, this 8e was submitted because of toxicity information for other endpoints. These were not addressed by this reviewer.

12b) 03 8EHQ-92-12709: Rank - medium.

Chemical: 2-ethoxyethanol (cellosolve: CAS# 110-80-5).

Draft NTP Technical Report on Toxicity Studies on Ethylene Glycol Ethers: 2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol (CAS Nos. 109-86-4, 110-80-5, 111-76-2) Administered in Drinking Water to F344/N Rats and B6C3F$_1$ Mice, US DHHS, dated December 1-2, 1992: Negative for gene mutations in Salmonella typhimurium in strains TA97, TA98, TA100, TA1535 and TA1537 both without and with metabolic.

Negative for gene mutations in the Drosophila melanogaster sex-linked recessive lethal (SRL) assay by feeding and by injection.

Positive for chromosome mutations (aberrations) in Chinese hamster ovary (CHO) cells in vitro without but not with metabolic activation.

Induces DNA effects in the form of sister chromatid exchanges (SCEs) in CHO cells in vitro both without and with metabolic activation.

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0192-12709 SEQ. A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Clorox Company

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0530) REFER TO CHEMICAL SCREENING
(0576) CAP NOTICE

VOLUNTARY ACTIONS:
(0601) NO ACTION REPORTED
0602 STUDIES PLANNED/UNDERWAY
0603 NOTIFICATION OF WORKER ENTITIES
0604 LABEL/MSDS CHANGES
0605 PROCESS/HANDLING CHANGES
0606 APP/USE DISCONTINUED
0607 PRODUCTION DISCONTINUED
0608 CONFIDENTIAL

SUB. DATE: 01/04/92 OTS DATE: 01/05/92 CSRAD DATE: 02/09/95

CHEMICAL NAME: CAS#

~~Diethyl~~ Cellosolve, butyl - 111-76-2

~~Methyl~~ Cellosolve, Methyl - 109-86-4

Cellosolve 110-80-5

INFORMATION TYPE:

		P F C
0201	ONCO (HUMAN)	01 02 04
(0202)	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04 ok.
0204	MUTA (IN VITRO)	[redacted]
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
(0213)	SUB ACUTE TOX (ANIMAL)	01 02 04
(0214)	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:

		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE RSQEST DELAY	01 02 04
(0224)	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
[redacted]	METAB/PHARMACO (ANIMAL)	01 02 04
0249	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:

		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEMPHYS PROP	01 02 04
(0244)	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
(0248)	PRODUSE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA: NON-CBI INVENTORY
(YES)
CAS SR NO
IN TERMINI

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
RAT
MUS
MOUSE
in vitro
DROSOPHILA

TOXICOLOGICAL CONCERN:
LOW
MED
HIGH

USE: Solvent, chemical intermediate

PRODUCTION:

2-butoxyethanol.

COMMENTS: Rats & mice were given the test Chemical at 0, 750, 1500, 3000, 4500 - 6000 ppm for 13 weeks. The major organs for toxicity were the testes in male rats and mice and hematopoietic System in rats & mice for these glycol alkyl ethers. (2 methoxy-2-ethoxy- and 2-butoxy-ether) 2-butoxyethanol reduced epididymal sperm density. The NOAEL is 6000 ppm in mice & in rats LOAEL IS 750 ppms

2-butoxyethanol